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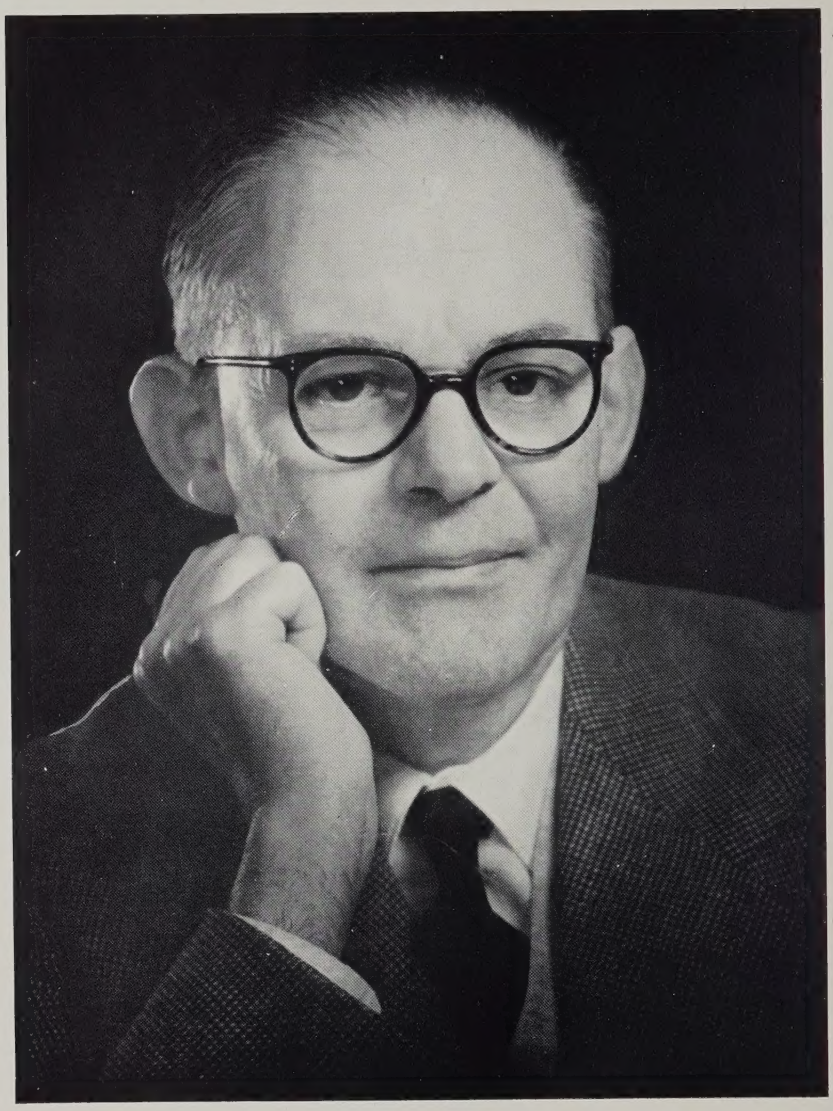

R. V. HARRIS

President of the Association of Applied Biologists, 1959–60

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THE ROLE OF RELATION IN APPLIED BIOLOGY

BY R. V. HARRIS

ADDRESS OF THE PRESIDENT OF THE ASSOCIATION OF APPLIED BIOLOGISTS DELIVERED TO THE ANNUAL GENERAL MEETING ON FRIDAY, 8 APRIL 1960

In selecting the subject of my address to-day I have assumed that the Association requires of a retiring President an account of some outstanding general impression he has received in the practice of his particular branch of applied biology and as a member of the Association which in his view may be of some interest and perhaps help to his fellow members.

The only qualification thus to generalize I can claim is some 36 years as a research plant pathologist, all of them at one fruit research institute in this country and as a member of this Association.

Over these years great changes have transpired both in my own branch of applied biology and in biological research generally. Thus plant pathology has emerged from being just one kind of applied mycology, bacteriology or entomology, to become a branch of applied biology in its own right quite comparable with that of animal pathology. And in the practice of biology generally we have passed from what might be styled an epoch of famous names to one of organization, specialization and a measure of anonymity. One could stagger the world *then* with a treatise on, say, 'potato blight and the potato famine', but not so much *now* with one on 'climate, ecoclimate and outbreaks of potato-blight'; or *then* on the 'control of apple scab by Bordeaux mixture', but not *now* on 'the significance of polyphenolic host metabolites in the nutrition of *Venturia inaequalis*'.

The fact is that from the occasional pioneer, often an amateur, questing in Promethian isolation and gathering round himself and his disciples an aura of startlingly new, indeed revolutionary, knowledge, we have come to research régimes in the various branches, with the scientists themselves at two-a-penny. We no longer tend to look up our references under the appropriate authority's name but in the subject-index of the appropriate abstracting journal.

First, such a changed situation obviously calls for an ever more complex organization of the body politic of research workers, if only to ensure that the various fields are properly covered and that uneconomic re-duplication of work is minimized. But can such quantitative organization itself ensure the maintenance of those high standards of creative originality and vitality associated with the great names?

No one had to worry about the applied mycology of a Berkeley or the applied plant physiology and horticultural science of a Thomas Andrew Knight. But has it occurred to you how infinitely the scope and productivity of two such giants might have gained if they could have been brought to work in association? But then, of

course, near-geniuses are notoriously bad mixers, or, as I prefer to put it, 'relaters' —they are worlds to themselves and there is generally no room for more than one in the same place, so that organization to this end might well have been frustrated in practice.

The incomparably enhanced potentialities of applied biologists working not only in contiguity but in positive-relation is my topic to-day. This phenomenon of positive-relation (and it is far more than personal) in both research and advisory activities, is, I am going to maintain, a vital condition for the attainment of highest quality and applicability in scientific work and, in a special sense, therefore, in applied biology, and most pertinently under the elaborated conditions of contemporary work I have indicated. The alchemy that this achieves when it happens on the personal level has indeed been a dominant experience of my own professional life and one that I am now trying to get out of my system for your inspection.

Unfortunately, however, as I have already hinted, vital relation in research is not commonly met; as with life generally it takes not too kindly to being organized. In fact relation is, in a sense that I shall suggest later, *the* primary organizing element within a research artifact. So, of another kind of artifact, pray who has ever effectively organized the painting of a great canvas except the painter himself? And again, one can only organize the single flowering of a *Welwitschia* plant by putting it in a desert in a tearing wind and waiting.

Let us first, then, consider in some detail what is implied by my use of the term 'relation', then its unique role, or rather roles, in our profession of applied biology, personal and otherwise, and finally, assuming that you are then with me in assuming that relation in that sense is indeed essential to the best research, what can or cannot be done to encourage it.

The nature of true primal relation

The metaphysic of Martin Buber (1937), Emeritus Professor of Social Philosophy in the Hebrew University, on the nature and exploitation of human relation has had a profound influence on the systems, or artifact worlds, of thought and action which man has created as aids to his survival, and not least on that of science, as one can readily see, for instance, in the work of that distinguished scientist and social philosopher Michael Polanyi (1959). The conclusions of these thinkers are, I suggest, of great relevance in the present context.

Buber distinguishes two basic movements which he claims are together characteristic of human living. The first is that of 'setting at a distance', corresponding in thought and speech with what he terms the *I-it* or subject-object confrontations of the ego. The second (in order of adult experience although both onto- and phylogenetically primitive) is the movement of mutual approach, of 'mutual entering into relation', corresponding with what he terms the *I-thou* form of mutual address. In the infancy both of the race and of the individual, man has not yet become fully aware of himself as a separate entity, but primarily of himself in relation with the world outside him, a world therefore not of objects, of entities, but of a shadowy co-inherence of being. In adult life this organic relationship with environment has

been largely lost and is only recoverable at times, and then discontinuously. If this were not so, the suggestion that our greater good (and therefore in this context greater efficiency as scientists) is in entering into such relation would obviously be meaningless.

We must indeed at first, and frequently thereafter, confront our research or advisory fields and their particular problems as congeries of discrete phenomena or entities to be judged, estimated, measured, criticized, defined and generally played-about-with, on the way to ultimate recording and description. But if our personal contribution is to be new and original, to extend and enrich our field of scientific knowledge, this kind of subject-object relationship must be regarded as entirely accessory and incidental to the other and primal one, in which the participants freely approach and indwell each other in an association in which the subject-object distinction is quite irrelevant. Such a relationship by its very nature, is, like life, unspecifiable except in terms of its result. That result is something uniquely new: *a creation.*

To enter into such creative relationship is in fact to combine the explicit knowing of the adult with the unifying intuitive knowing, or 'innocent eye', of the child, and such moments of full mutuality are indeed our moments of vision, moments of total synthesis, moments of unvarnished truth; I say 'unvarnished' advisedly because to equate reality with the world of 'it' or the purely objective and therefore potentially oppositional is in self-defence to exchange truth for a purely artificial phantasy or hide-out for a disestablished and solitary ego, romantic, existentialist or what-not. 'In the beginning is relation', postulates Buber (1937), and it is in that positive sense that the term 'relation' appears in my title. To avoid any possibility of confusion, however, I shall hereafter distinguish that primal positive relation by the epithet *creative* and the secondary I-it or descriptive relation as *particularization.* The relations of the practising applied biologist as a single worker will further be distinguished as *internal,* where the co-participants are the immediate subject and experimental *materia* of his work; *personal* where the co-participants are his fellow scientists and other associates.

To-day I am ultimately concerned with the latter, with the potentialities of the personal relations in this sense, but as these arise from a situation determined by the primary or internal relations I must briefly discuss them first.

The creative internal relations

I have referred to as first in the sequence of adult experience that attitude of the scientist when in tackling his problem he proceeds to fix its outlines by confronting it at a distance—in short to define and describe it—the confrontation that Buber refers to as the I-it tension. But, to regard this attitude, with the empiricists, as an end in itself and as the sole ideal in scientific method, to regard all which is not expressible in explicit and impersonal terms as dubious vestigial and unscientific, as a fallible 'human' survival into science and therefore something ultimately to be eliminated, is I suggest totally unsound and fallacious.

Thus, for example, long before the specification of a motor-car has been carried to a description of it in the ultimate terms of its electronic or molecular structure, that specification will have lost all working relevance for the engineers who have to create the car; one constructed on such a 'map' would most likely be quite ineffective as a means of transport. Push beyond a certain point, therefore, the process of analysis, of data-collecting, of pulling an entity apart to find how it works, and the scientist will lose all effective touch with his entity, with the 'whole' which is his primary concern as such. To change the simile and borrow again from Polanyi (1959), 'Take a watch to pieces and examine, however carefully, its separate parts in turn and you will never come across the principles by which the watch keeps time', or again, to come closer home, section, examine and describe an infected root in its most intimate associations of host tissues and pathogen *in situ*, and you will never describe the disease thereby *alone*. But having achieved a firm grasp of the face of the disease as a whole, having comprehended it, then such analytical studies of its particulars, even into fields involving biochemical or physiological approaches, can do nothing but enrich that primary understanding.

The fact is that although our intelligence naturally tends to move first to analysis, definition and description, our personal instinctive grasp of the whole field of our problem, our positive relation with that 'whole', is both primary and ultimate, the absolute prerequisite and end in our practice as biologists. Again, according to Polanyi (1959) all meaning (and, I would add, therefore significance and applicability) in scientific knowledge lies in and springs from this purely personal faculty of 'comprehending in a relational act a set of particulars in terms of a coherent unity'.

Now this is just where creative relations of the kind I have mentioned come in, first in what I have called the internal relations of the scientist in his work. These, I suggest, are a threefold complex which I think is most clearly exhibited by the corresponding internal relationships of a practitioner in another creative field of human activity, *the visual arts*. Here as a result of a creative relationship between the artist and an external object, his subject, say an apple tree or a piece of country, on the one hand, and, as an understanding and practised craftsman, with his media on the other (e.g. his canvas, priming and pigments) an unique creation, a tree which is both more and less than that original tree, or in the other instance a landscape, *in terms of his medium*, is brought into being, and this in time may command a fabulous sum of money at Christie's. This kind of creative happening, although it may be encouraged or even inspired by the artists's working associations and environment, cannot be produced to order, cannot in fact be *organized into being* by anyone or any system other than that inherent in the personal creative complex itself. The artist by entering by volitional urge into mutual creative relations with his subject and the medium in which he is working, brings into existence thereby a new extension of total being which is the canvas, sculpture or what-not, itself in its turn a vital begetter of similar creative activity in those who are tuned in craft or in connoisseurship to relate to it.

Substitute for the visual artist's subject say the apple-scab disease or a fungal

mould, and, for his medium the plant pathologist's and mycologist's apparatus and conditions of experimentation (with their determined and accepted limitations) and that act of threefold interrelation will produce an extension of scientific knowledge, a new hypothesis, the very creative stuff of our craft as scientists, as applied biologists.

Such a personal tacit creative relationship with the subject and medium transforms the particular piece of 'country' with which that scientist is concerned into a unified whole or pattern, for example the comprehension of an apple tree and the fungus *Venturia* in that particular mutual dynamic relation which *is* apple scab. Everything that is thereafter done in producing the finished scientific record of that relation, the 'map' of the disease, for the use of the plant pathologists and advisers generally, to aid the production of sound apples thereby, will emerge successively and explicitly from the progressive enrichment of that primal pattern which is apple scab with ever more significant detail, with those effective particulars determinable within the existing limitations of scientific technique and experimentation. As a result, a living chain of experimentation, hypothesis and counter-hypothesis is started, the product, at each stage, being a map of apple scab ever more closely approximating in communicable terms with the real entity which is the disease itself, and bringing the disease therefore more and more within the control of man.

Alternatively, if the starting subject is one in the so-called field of pure botany, such as, say, fungal moulds of a particular genus, the ultimate focal aim of bringing the world of moulds within human control is further removed, but only in the sense that, for example, those motor engineers whose attention is focal on the carburetter are only one stage further removed from those focally concerned with the complete motor-car; go one step further still and assume the focal field to be the physical and chemical properties of oils, and then no one working from such a field or standpoint *alone*, however elaborated, would be likely to produce within the span of human persistence a formula for a complete motor-car, however dependent the *running* of the latter may be on oils. Such research therefore might well provide the engineer with information by which he could greatly improve the performance of that car. Thus a study of moulds *per se* might seem far removed from the discovery of an aid to human survival, but the fact remains that it was a diversion of focal attention to certain particulars of fungal moulds, brought about by a pure accident in a piece of routine pathology, that ultimately produced penicillin. If that scientist's attention had been exclusively fixed on his routine bacterial culture operations and he had not therefore achieved an intuitive movement within his primal and wider focal field in pathology to a subsidiary awareness of a phenomenon in the field of pure mycology and bacteriology and its possible implications in his pathology, that great discovery would never have been made, far less consolidated as we know it to-day.

Now you may begin to see that my earlier-drawn analogy between creative scientific practice and the visual arts was not entirely fortuitous; and in fact I find myself in the best of company in actually regarding such practice as one of the arts, and a good scientist as a good artist in this sense. The discovery of penicillin was in fact a work of sheer artistry. Thus an outstanding manual on the requirements and

planning of scientific, and in particular biological, research is entitled *The Art of Scientific Investigation* (Beveridge, 1950) and Vick (1959), Professor of Physics in a Midland University and a member of the University Grants Committee quoted, in a recent broadcast, Sir Cyril Hinshelwood as saying 'Science is an imaginative adventure of the mind seeking truth in a world of mystery' and then proceeded to protest at the popular impression of physics generated in schools of to-day as a collection of facts regarding the properties of matter, or of botany as the classification of plants, and so on, coupled with a vague notion that science is that which has given us television and atomic bombs. 'There is little understanding,' he goes on, 'of how much these developments have depended on the principles and concepts created in the minds of scientists; and I mean created.' Surely an authoritative whack at the purely empiricist view of our science; for the ability to create is, humanly speaking, I would suggest, the ultimately distinguishing attribute of the human species, and the crucial and unique instrument, however misapplied, in man's fight for survival in a world where that supreme faculty has become fragmented, almost beyond recognition.

THE CREATIVE PERSONAL RELATIONS

We have so far considered the internal relations of a creative scientist in his work as the functional unit in our profession. But a scientist is also concerned and associated with colleagues in research, advisory work or in problems of application or administration. If this were not so the existence of this Association would have no point or meaning, and it is at least assumed that a researcher will take the precaution of occasionally referring to published work by others on his subject.

This personal factor receives scant if any mention in handbooks on research method such as the work I have already mentioned, possibly because such entering into relation cannot by its nature be specified; it just happens or not. The willingness or capacity to do so in a candidate is probably more elusive, even, than is a creative faculty and urge for individual research, although these qualities are, in my experience, frequently found linked.

And yet, as Dr John Ziman of King's, Cambridge, remarked recently in a witty broadcast (1960), scientific research these days is being transferred from its original status of a voluntary activity of gifted individuals, the traditional scientist, with a bent that way, to that of a highly respectable, if not wildly lucrative, profession among the other great professions, requiring only certain academic certificates for a licence to practise.

Research careers must needs therefore be scheduled and organized in all the appropriate stages from school onwards, the impetus for entry being generally a romantic illusion such that qualification as a scientist is a passport to ride round the moon in a rocket!

Having finally been appropriately dubbed thus, the new research scientist will then find himself stranded in a laboratory, far from alone or allowed just to select and follow his own bent, however appropriately 'pure' or 'applied'. He must

perforce now work on a pre-determined problem, at least nominally, with or alongside colleagues with the same Ph.D.'s, in the particular department or group to which he has been assigned; or die.

Of the present economic necessity for thus mass-associating applied biologists there can certainly be no doubt, as also for their assignment and organization. Thus for such purposes one may, for instance, situate say an entomologist with a plant pathologist, but you cannot thereby make them relate.

One of my distinguished predecessors in this Chair two years ago devoted his memorable Address to 'The organization of research' (Findlay, 1958), but the references he made to problems raised by personal relations, or the lack of them, were indirect, as when he commented on the fact 'that switching people around within a staff always caused some distraction and possibly even resentment'. He mentioned also, critically, 'the central recruitment of scientific staff practised by the Civil Service', reminding one that our National Agricultural Advisory Service moves its scientific staff to new geographic and often agriculturally diverse districts in the normal process of promotion, with the disruption of the adviser's delicate and hard-come-by relations with his district.

Now, as I have already suggested, the quality of vital relation which a scientist has to establish with his scientific field if he is to function creatively, is of the same kind as that which can relate two scientists in their different fields. It is the same kind of mutual moving-towards, of mutual indwelling, which creatively relates the scientist jointly with his field of reference and with the appropriate resources of his profession for work in it; to his experimental *schema* in short. There, however, it is the interrelation of the man, his inarticulate subject and his medium, here it is the interrelation of these primary relational units themselves, a vast extension of scientific potentiality.

Perhaps we can best get the clue to the nature of this personal relation by a glance at the advisory field. Here one of the elements in the adviser's primary set-up, his medium, is in fact not first and foremost, as in research, his experimental *materia*, but with the *persons* he is to advise and consult, with his farmer or his grower and with his research associates. This relationship must be something very much more than an exchange of advisory bulletins or vague assurances of co-operation. Good and effective advisory work is an art, a creative activity resulting on the one hand from vital relations with that adviser's branch of agriculture including its research practitioners, and on the other with the farmer, his particular farm and his specified and unspecified problems. The latter relation must be such that each participant, adviser and farmer, brings his own unique contribution in knowledge and skills to a mutual indwelling of each other's understanding of the problem in question, the result being the improvement of the adviser's equipment as such on the one hand, and the farmer's practice on the other. Each will see in the single advisory situation what the other from his standpoint alone could not, and bring that contribution to the joint completion of the whole picture.

Now let us return to personal relation in research. Say, for example, that a pathologist and an economic zoologist are each concerned with work on the same

crop. Both are at an institute concerned with research on that group of crops and therefore have at their disposal as the *materia* of their researches not only the special resources of experimentation adapted to the pathogens and animals constituting one side of the primary relations, but with all the field and reference facilities of an institute thus concerned with *all* aspects of the economic production of those crops. Each may find that he is in fact working on the same experimental plot on associated disorders of a particular crop plant, but for one the subject or raw material of his research, the piece of country with which he is interested, is bounded by an interaction between his crop and his animal pest, the other between the same crop and his plant pathogen or potential pathogens. Each, although thus physically associated in work on disorders of the same crop plant may well be relationally isolated with only objective fortuitous and incidental interests in the other's problem. Each will produce one new hypothesis at least on the causes of the disorder he is studying, interesting as far as it goes but not unfortunately a completely efficient instrument in controlling it.

Then these two scientists *really meet* in the sense that each steps within, comes to indwell, the world of the other's problem, each thereby bringing to the common ground thus established his own personal contribution derived from his own primary relations with that problem. And thus the alchemy occurs, and these two individuals come to work as one freely constituted mutuality with the result that two limited spheres of reference are transformed into one common greatly enlarged focal field embracing, as one, the worlds of the animal pest and the pathogen. The possibilities inherent in the study of what is now one shared problem are thus immensely multiplied and, for instance, it may even emerge that the relational structure of each disorder is not after all twofold, but threefold; pathogen, animal and host. One could multiply examples of this kind, and of course such associations of workers of diverse disciplines can be extended indefinitely, the products becoming not a string of studies by individuals, but a related complex by a voluntarily related multi-personal organism (not organization) with appropriately and infinitely enlarged and enriched scope, both in depth (or specialization) and in the extent of the country coverable.

The quality of the relation between these naturally associated workers will ensure that however deeply into the *minutiae*, or particulars, of that problem or field of research each may delve, his own investigation will never become an end in itself; he will never lose his focal awareness of the whole field his group is covering and will therefore never find his line of research losing relevance with that field. He will never have difficulty in knowing where to start in and where to stop off.

Further, this creative relational personnel is of course not limited to research only. As the rolling snowball it will gather up the imagination of advisers (with their own special skills and creative fields of reference) of the producers and of the administration consolidating, codifying and making generally available the products of such joint research and advisory activities. Here I may just say that the fiction of the concordance between the entomologist and the plant pathologist I have made is founded on fact for it has happened at least twice in my experience,

once in the resolution of the raspberry midge blight complex (Pitcher & Webb, 1952) and again in the discovery of the dual, nematode-bacterial, origin of the so-called strawberry cauliflower disease (Crosse & Pitcher, 1952).

I have claimed that one could multiply such actual examples of the effectiveness of personal relationships in applied biology, but to-day it must suffice if I briefly recall three large-scale combined operations in plant pathology, in which I have been privileged to participate in some small way.

(1) Success hitherto in the campaign against the ravages of progressive *Verticillium* wilt of hops in the south-east has absolutely depended on just such creative relations between research and advisory staffs in hop pathology and on the hop crop itself on the one hand, and on the other with the hop growing and the brewing industries, and the appropriate plant pathology and administrative staffs of the Ministry of Agriculture, Fisheries and Food (Burgess, 1956).

(2) The elaboration of fruit plant certification to embrace experimentally determined standards of freedom from important pathogens has involved the same imaginative and creative working-together between the research, advisory and administrative arms in horticulture, together with help and support, moral and material, from pioneer spirits in the relevant industries themselves (Harris, 1954).

(3) The recently launched 'operation fire-blight', the effective conduct of which has depended on swift and elaborately planned action on a nation-wide scale, has been possible because of the complete mutuality which has developed between the arms involved, namely the relevant Research Station, the National Agricultural Advisory Service and the Ministry's Plant Pathology Laboratory and Plant Health Branch, with the full co-operation of the fruit-growing industry (Crosse, Bennett & Garrett, 1959).

But finally, it must be faced that such creative personal relations are by their very nature most sensitive to disruptive influences. Anything in fact which contravenes the tacit assumptions of integrity and *bona fides* in entering into such creative mutuality must prove mortal, for, as Polanyi (1959) suggests, it is by an act of mutual appreciation that we enter into a fellowship with an associate (in this sense) and acknowledge that we share with him the same firmament of obligation, personal and scientific. This, he goes on to say, is how we come to understand it and accept it, that he is in his work a person capable of responsible choices.

The existence of such a tacitly assumed basis of mutual confidence is the natural guarantee of two particularly important conditions for the maintenance of joint creativity in research of the quality I have described, namely (1) a mutual just apportionment and acknowledgement of credit in the results attained in the joint work, and (2) the maintenance of absolute candour between the participators. Regarding the latter especially, when candour is replaced by concealment by an associate for his own supposed benefit, at that moment that associate voluntarily steps outside the relational sphere, mutual trust goes by the wind, creative association is destroyed, and our science, in the long run, will suffer out of all proportion to the purely numerical dimensions of such a disruption.

The complementary role of particularization

So far I have deliberately, in conformity with my theme, stressed one only of the alternative movements of the scientist's mind in face of his phenomenal world, the positive relational and creative one.

The other, the setting of phenomena (including our colleagues!) at a distance to determine their lineaments, is no less important but, I am going to suggest, only in a complementary and accessory sense. It is in fact the movement of consolidation which follows the pioneering relational expedition, that primary comprehension of our total research field.

The two kinds of knowing corresponding to those two attitudes can be referred to, in Polanyi's terms (1959), as *tacit* for the former, the personal contribution, and *explicit* for the latter, or particularizing movement. Tacit knowledge involves, I have suggested, a personal participation or, again to quote Polanyi, an indwelling, either directly in the phenomena we are studying or relationally in the comprehensions of our colleagues, or, indirectly, in the body of explicit or objective knowledge we have at our disposal; 'the knower participates thus personally in shaping his knowledge'. The explicit documentation or expression which follows is the end and tangible product of the whole operation. To put it precisely, tacit knowledge is comprehending the parts as a whole; explicit knowledge is the particularization of this whole in terms comprehensible or communicable to other scientists. Explicit knowledge is, one might say, a critical description of the country we have discovered, the map we have made as a result of our tacit relations with it. As such it is public property and thus open to critical examination by all and sundry, for a map may be misleading.

The production of explicit or formulated knowledge is therefore an essential function of scientific activity but its practice and products are not ends in themselves. The map will never be a complete substitute for our personal knowledge of our bit of biological country. But by the combination of the two, by the production and use and improvement of the map, combined with the application of our personal or tacit faculties for comprehension, we should be able to practise our science in the fullest and richest sense. Once again, it is all a matter of relation, here between the tacit or personal and the explicit or particularizing approaches to knowledge.

The relations of pure and applied biologists

Now, having, I hope, obtained some grasp of these two complementary approaches to our problems and our colleagues, the primary one of creative relation, and the accessory one of particularization, we are in a position to attempt a specific application.

I have already used the distinguishing terms (borrowed from Polanyi) focal and subsidiary as describing two kinds of tacit awareness or understanding of the phenomena we study, and have hinted that once our focal attention expands and we become aware of the phenomenon or entity we are working on as part of a larger

comprehending 'whole', that awareness of our problem is thereafter different, it has become subsidiary instead of focal, although we may have to revert to focal attention at times as it becomes necessary to give subsidiary attention to *its* parts.

Thus a focal understanding of, say, apple mildew would be enriched subsidiarily were we to discover particulars of the *modus* of that fungal invasion of apple foliage, but would become enlarged if the expression of the disease were comprehended as an incident in a particular nutritional unbalance of the tree. The nutritional relations of trees are of course focal for some horticulturalists, but for the pathologist his understanding and approach to work on this disease would, after such a discovery, have undergone a fundamental change. To have arrived at such a conclusion from focal attention limited to the disease only and the consequent analysis of its particulars, would probably never have led independently to such a conclusion, that is unless indeed the deficiency of an element in a tree's make-up were related *directly* to the nutritional requirements of the pathogen. For a pathologist thus to experience the transference of his awareness of his specialist problem, here apple mildew, from focal to subsidiary is by no means retrograde. It implies in fact that his problem is gaining its true relatedness as an incident on a wider stage, and not as the play itself. Therefore we see that real creative progress with our particular problem must ultimately be motivated and directed within a focal awareness of our subject in its widest 'whole' or context. This, in the example of apple mildew we have quoted is the productive apple tree. It is as with the artist confronting his piece of country. He will never selectively transform this in his creative being into a unity, a pattern, a landscape, in terms of his medium, until he has organically, in the I-thou sense I have described, achieved complete relational mutuality with both, the tangible result being that new 'whole' or extension of being which is the landscape painting. It may start on his canvas as a flurry of interrelated curves and cubes and surfaces; the detail will surely grow into the completed whole, and in the process, every now and then, he will stop painting, stand back, and examine that canvas critically over against the original subject.

Now it may begin to be evident why rational definition, analysis, classification, accumulation of data and all the rest of that kind of thing, regarded by the empiricists as the beginning and end of scientific work, is, applied as an end in itself, the very negation of the scientific art. Nothing creative will ever come from such an approach alone.

Definition in this total ultimate sense is something to be avoided for it is antirelational, and without relation no new creative artifact will emerge. Resolve a focal unity into two particulars by all means but not thereby to split irreparably that unity; only as a means for specifying those parts in order to enhance our grasp, and thus our control, and use of, the whole. To lose sight by critical analysis or enumeration, of the whole, can thus be destructive both of meaning and direction in our work. It will destroy the whole by inducing a mutual opposition of the parts. And yet, you will find yourself, that as soon as you define anything, i.e. set it against its opposite (outline delimits) you will tend to regard the two resulting concepts as not only separate but *opposed*.

Now two kinds of biology are popularly distinguished, pure and applied. If we are 'applied' we are presumably, by definition, impure and vice-versa. On this assumption you may detect an unmistakable nuance when a pure biologist refers to that other kind of biology and its practitioners (you and me)!

As an 'exercise in relationship' (with apologies to Mr Stephen Potter) let us now apply the other way of confronting such distinctions in scientific thought, the way I have suggested, the relational way.

Let us first be honest about the aims of the scientific discipline and of biology in particular, as a whole. Like a hammer it is not, of course, an ultimate or absolute end in itself, and therefore its practitioners cannot be regarded as ultimately disinterested, or ultimately 'pure' in their intentions, no more than, we hope, can the hammer's designers or wielders. True connoisseurship of hammers can only spring from hammers in use. When we are true to our true-selves, and true personality according to Buber is only realized in real relation with the phenomenal world, we therefore see science as part of a much wider 'whole' as one of the many systems of knowledge created by man, in his bid for survival, to aid his survival. All science is therefore 'a hammer' and in this sense 'applied', or, to change the metaphor, all science is *one* of the maps made by man to help himself not to get lost in his real world. What he must not do for a moment however is to *identify* that map with the real world, for it is only *one* of the communicable and inheritable instruments he makes and bequeaths to aid his terrestrial control. The *practice* of all science is therefore a craft, i.e. man's art of making something for his use, and as such 'applied'. Applied biology is therefore actually *primary* to, and inclusive of, pure biology.

Here, once more, the visual arts are I think illuminating, for here in the West a similar distinction is made there, a distinction between fine or pure, and applied, art. This, as in the corresponding 'pure biology', may be functionally and economically convenient in permitting in this case a certain section of the artist community to doodle indefinitely and lucratively, without any utilitarian object except in facilitating the doubtful practice of superimposing framed canvasses of assorted sizes and merits on our wall surfaces, with most indifferent and conflicting results. But, of course, to glorify these so-called fine-artists at the expense of the humbler applied ones, represents the extent to which this distinction has issued in a quite unreal opposition. For surely if the arts have any purpose it is to make human living, at least as far as its inanimate context is concerned, more gracious and liveable, and that is just applied art; not for instance to throw the essential ugliness of a Victorian domestic décor into stronger relief by hanging good pictures! The visual arts therefore, like those of biology, are essentially one and indivisible, and essentially 'applied'. It is further significant that only in the applied arts is effective collaboration between the different kinds of artist to be found, as for example in architecture where even the fine arts are brought into the team—another example surely that the wider your focal awareness or scope, the greater the need for spreading the load of work by appropriate personal relations between the subsidiary fields of activity, pure and applied.

Thus, assuming that all science is ultimately an instrument or a map for man's use, i.e. that ultimately awareness in all scientific activity is subsidiary to that of the greater entity which comprehends it, human existence, nevertheless in making, say, a hammer, and for that matter in making a map, lots of subsidiary jobs are involved which, to ensure proper efficiency, require themselves the focal attention of the workers. Thus makers of hammer-hafts must work, at least at times, as if their professional existence begins and ends with hammer-hafts.

Now come back to the example of apple mildew. No one would deny that work on and against apple mildew is applied biology, and we have seen that although the plant pathologist may start the work on it, he may be naturally excluded from certain bits of it by the simple process of transferring his focal awareness to a more embracing entity within which the apple mildew disease as an entity can only command subsidiary attention, to a point when the horticultural scientist could, if he were foolish, dismiss apple mildew as a convenient and even desirable symptom of some elemental deficiency in his trees! Let us now travel in the reverse direction, this time in the contracting ranges of focal awareness. When the plant pathologist starts to work on apple mildew it is not long before he finds that the disease is the result of the interaction of two plants, a fungus and the apple tree. Now, he would be hard put to it if, having found the fungus pathogen, there were no key, no map, no fungus flora by which he could identify it. Of course there would be, and that map is the product of a scientist who, to make it, has established his professional relations primarily in a *world of fungi* and their substrates, in this case the world of the parasitic Erysiphales. But in his work as a pure mycologist that scientist is in fact making a map of a tract of biological country, perhaps obscure and hitherto untravelled, which may well prove, as in this example, to be invaluable to his 'applied' brethren. And it is not entirely unheard of, I may say *sotto voce*, that a plant pathologist himself, 'applied' though he may be, *may*, in the course of his studies of his pathogens in relation even contribute, himself, to the pure biologist's key to the fungi. He may even have to make descriptions of his pathogens unaided by the latter, in doing so achieving lightning metamorphoses in the roles of both pure and applied biologist! Only in so far as he might lose his bearings in such excursions into the pure realm and find himself thereby unable to regain focal awareness of the mildew disease, could he as an applied biologist be said to be trespassing in the 'pure' field and thus using his time uneconomically!

So you see we conclude this game of relationship in applied biology firmly convinced that any suggestion of opposition or mutual exclusiveness between 'pure' and 'applied' there is a fiction without foundation in fact. Both categories are just working conveniences, and both are basically and in origin 'applied'. But some of the 'pure' practitioners must of course conveniently forget this for the purposes of their own essential part in the relational programme in which, in the particular example of apple mildew, pure and applied botanists and mycologists, with, when we come to the ultimate problems of control, chemists and physicists, in the advisory and research arms, and, very importantly, the farmer himself, are relationally involved. In this light biology is ideally one complex integrated instrument of

man's survival, with two main modes of practice depending on whether we are making that instrument or using it, and conveniently, although very roughly, dubbed 'pure' and 'applied' respectively, each kind of practice losing all significance as soon as it is regarded as an ultimate end in itself; as with the hammer.

Conclusions and applications

I have attempted in the short time available to expound and illustrate the views I have come to hold on the vital role of positive creative relations in all original scientific practice worthy of the name, and as I have also suggested, in applied biology especially. There is still of course a welcome place for the relatively solitary 'old-world natural scientist' particularly in pure biology. But in applied biology, as connoted to-day, there is everything to gain in encouraging work in freely associative and distributive groups; in fact only thus, I feel, can the growing necessity for deeper specialization be reconciled with the greatly enhanced extent and complexity of the ultimate problems of control which confront us, only thus can the vital relations of the specialist with the whole territory within which he is working be maintained.

Assuming this trend towards the 'cell' or group of workers, I have maintained that the vitality of such a group will be solely derived from the associative dispositions of its members as free persons in creative relations with each other. How then, finally, can we organize for such associative work and at the same time ensure that the 'bodies' thus produced are not rendered moribund thereby?

On this, the application of our findings in contemporary educational and administrative practice, I have only the time to offer the broadest hints and generalities.

I will first assume, therefore, that we are in general agreement on the main premises and conclusions I have tried to maintain, which are briefly as follows:

(1) In the arts, creative relations of the practitioners with their subjects and media, and, if execution is collective, between themselves, are the vital necessity.

(2) The practice of applied biology, as indeed of all science, is an art.

(3) Therefore the effective practice of applied biology implies the prior establishment of relations of the same creative quality, both internal between the scientist, his field and his experimental *savoir faire*, and personal between the appropriate co-workers.

The present problem therefore becomes, in short, how to produce scientists not only academically knowledgeable but qualified also to *practise* creatively in however small a way, anonymity being the natural home of the pure in heart and intention, provided only that his powers are fully engaged.

The converse of anonymity, like Jules Verne's Captain Nemo, implies a romantic view of one's vocation, a screen from the reality which even to name is surely to die. And *first* with Dr Ziman (1960) I feel that such a view of science as, for instance, a glorious passport to the moon should be debunked in the school stages. Science should be taught as, ultimately, a sober and useful, and even a noble and sacrificial, art or craft, requiring as such do, much learning, but then unlearning and laborious practice under skilled Masters in the medieval sense.

Secondly, adequate facilities should be available to turn an academically qualified student into a professional applied biologist, a very different kettle-of-fish. And on our present assumptions the basic qualifications here are an aptitude and natural urge to establish creative relations within his field and with his associates and, armed with adequate scientific training and equipment, to be able thus to expand his focal awareness from his immediate problem to something which can only relationally be solved, something both more complex and comprehensive whose fascinating working can only be jointly mastered.

To achieve this end it should be generally admitted that a potential professional in science has far from finished with his training when he has graduated from his university, but that it is at precisely that point that the crowning part of his education as such should be starting; whether it be continued at a university or at one of the research or advisory centres being decided by his particular bent. Certainly in plant pathology I suggest that the crop research institutes are uniquely equipped and situated to do this, for reasons I have hinted, for there, from the start, his primary interest in the diseases will always be subsidiary to a focal awareness of the crop; in short the frame of the picture will be sufficiently comprehensive to ensure the growth of proper internal and personal relations in his specialist work.

Wherever he may be situated for his post-graduate training, however, the point is that *that training is going on* and that he is therefore not to be expected at that stage primarily to produce, but to learn and *acquire* the art of research, or of whatever his own particular function in the profession is to be; he is not merely there to 'do postgraduate work', but is on a *postgraduate apprenticeship*, and the vital key to success with such is of course the calibre of the Master (in the medieval trade-guild sense) who is to transform the merely informed into accomplished practitioners, largely, as I have said, a process of *unlearning*. Here I find I have used the precise term, *apprentice*, adopted in the context of a prevocative report just published jointly by the Institute of Physics and the Physical Society entitled *The Postgraduate Training of Physicists in British Universities* (1960).

This master of apprentices should be one not only qualified academically, but, as essentially, with a capacity to inspire or activate creative relations with and among his students, firing them thus with the necessary urge to do likewise with their material and their associates. Only a creative relationship between master and apprentice will produce, in the apprentice, as we have seen, the basic tendency to relate, where relative anonymity, as in the medieval guilds, is a positive advantage. The master will be primarily concerned to put his student through the mill of acquiring the 'mystery' which is his craft (and it is indeed a self-testing, and at times despairing, mill) not expecting to earn by his student's hard work an augmented output of publications under his own name! He will set his student alight so that he can, Zen-like, get to use his sword like a swordsman, that is without having to think about it whilst in action. And incidentally an adequate apprenticeship in this sense should provide that vital statistic on a graduate (over and beyond references from his graduating school) which is needed for successfully directing him to, and appointing him in, his first job.

Thirdly, comes the problem of the first appointment, and here I am absolutely in agreement with my predecessor (Findlay, 1958) in concluding that placing the right man in the right post in an Institute should not be the job of a central authority. The Institute itself should advertise informatively its vacancies and should interview its own candidates. And even then, assuming that all other qualifications are suitable, the intangible genius to relate may elude the interviewers. But of course here we shall now have the great help of the master of the postgraduate apprenticeship, with his personal data and conclusions on the man.

Fourthly, having engaged the candidate, it is then vital I suggest for his subsequent scientific health and output to place him within an already creative environment of research associates, and I think we can assume that this will be practicable if all of them, or a majority, have been similarly selected for relational and creative qualities.

Finally, our applied biologist having been settled in a context in which he will readily adapt himself and respond in the relational sense, are there any aids to keeping him happy there? The best assurance of such continued effectiveness is in the degree in which the focal attention he has to give to his particular line of investigation can become subsidiary to a live appreciation of the wider contexts of his field; in other words, in whether he continues able to reassimilate his part into that whole which is his ultimate field of reference, his job in fact.

There are indeed such 'aids' and one of them is our own Association. For such an Association of professionals provides the perfect antidote to the dangers of over-specialization (which can be briefly summed up as professional isolation) with its open forum in which we can professionally relate, even in finding ourselves in friendly differences, with diverse workers in our diverse fields of applied biology. The crux of such a benefit lies precisely in the breadth and variety of the topics which are discussed at our meetings and also from the opportunities for free discussions of these and others, ranging far and wide, not only in the organized meetings but informally before and afterwards, in the lobbies, at lunch or at tea. For me the latter, and most informal, parts of our meetings with colleagues are possibly at least as helpful as the organized discussions, tending as the latter must do at times to become somewhat stilted and formal, probably because they *must* be organized. I personally try to counter with all my might the trend these days for members to attend only those sessions which concern their own immediate subjects, or again, proposals to hold in place of general meetings simultaneous ones of small specialist groups, rather than more and more broadly based meetings and symposia. These trends, in my view, tend to counter that unique benefit our Association has to offer which is, in short, to stimulate the awareness of the more specialized by extending the range of their focal attention within the whole field of applied biology. It is so often in listening to papers and discussions on branches of applied biology remote from one's own immediate specialist field, or at least remote from fruit pathology, that one has gathered ideas for one's work, and clues to developments actually implicit there but hitherto obscured by over-familiarity and, as I have said, uncompensated specialization. Quite apart from their strict or applied

utility, such meetings do certainly provide all the general benefits of a change of scientific air!

I continue to remember an *obiter dictum* of William Brierley, when he was Secretary of this Association, at a General Meeting many years ago at which this Association's aims, objects and programme were, as again this year, under discussion. He summed up the unique associative benefits conferred by our Association on applied biologists as 'Coffee at eleven' and I would add, I hope good coffee at that! If, as sometimes with our immediate research associates, we can stimulate at our meetings free, reciprocal and uninhibited interchanges between applied biologists of the most diverse disciplines, we shall have contributed much I suggest towards keeping highly organized and specialized scientists sane and green, keen and effective, in their work.

Dialogue is Buber's term (1947) for such creative, in the sense of truly mutual, verbal relations between persons. We unfortunately use the term 'discussion', and more informally just 'conversation'. *Discussion* is, of course, a kind of organized talking which, however, in practice too frequently turns out to be a series of rather defiantly delivered sub-paperettes. Buber (1947) refers to 'that curious sport aptly termed "discussion", that is, "breaking apart", which is indulged in by men who are to some extent gifted with the ability to think'!

And of *conversation* M. de La Rochefoucauld (1665) has a pregnant maxim (no. 139) which has been felicitously translated by Mr Tancock (1960) thus:

> One of the reasons why so few people are to be found who seem sensible and pleasant in conversation is that almost everybody is thinking about what he wants to say himself rather than about answering clearly what is being said to him. The more clever and polite think it is enough simply to put on an attentive expression, while all the time you can see in their eyes and train of thought that they are far removed from what you are saying and anxious to get back to what they want to say. They ought, on the contrary, to reflect that such keenness to please oneself is a bad way of pleasing or persuading others, and that to listen well and answer to the point is one of the most perfect qualities one can have in conversation.

One could well apply this exposé as an indicator of one's capacity to relate. Truly, as Buber says, 'all real living is meeting', but meeting in the creative sense I have tried to exhibit.

Let us, as applied biologists, aim to nurture the art of relation in our own work and in that of our colleagues, for I can vouch that whether one merely sees this happening in others, or, better still, participates oneself, there are few, if any, experiences in our kind of work more exciting, satisfying and rewarding, when it happens. Any fool can compete!

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THE EPIDEMIOLOGY OF *PHYTOPHTHORA INFESTANS*
I. CLIMATE, ECOCLIMATE AND THE PHENOLOGY OF DISEASE OUTBREAK

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(With Plate 4 and 7 Text-figures)

Temperature and relative humidity of air within and above potato crops were recorded throughout the growing seasons of 1951–8, with wet- and dry-bulb mercury-in-steel thermographs. Diurnal periodicity curves of successive weeks showed that conditions within the crop changed, relative to those above, as the crop grew. When much bare ground was exposed, the air within the crop was warmer and less humid at midday than at 4 ft. above ground, particularly in dry and sunny weather. The growth of the foliage and the amount of sunshine and rain determined how quickly this pattern was replaced by one with smaller temperature differences and crop humidity slightly in excess of that above it, at all times of day. Rain on crops with dense foliage almost completely eliminated temperature differences and gave relative humidities over 90 % that persisted in the crop for a larger proportion of each day than in the screen. Finally, as the disease defoliated the crop and again exposed the soil, the relationship partly reverted to that early in the season.

Above the crops, curves of the number of hours per day with relative humidity not less than 90 %, rose and fell with the passing of wet spells. At first this was also true within crops, but when foliage was dense the rise in relative humidity after rain often persisted through intervals of dry weather. Such conditions usually preceded the appearance of potato blight in epidemic proportions by 1–3 weeks. It is difficult to account for the regularity of blight outbreaks by seasonal climatic changes, and although many factors are probably involved, changes in ecoclimate seem important. When knowledge about the response of *Phytophthora infestans* is related to the meteorological observations and to the dates of blight outbreak, the growth of potato crops seems often to be self-destructive, because it progressively modifies the immediate environment to favour the spread of the crop's most destructive pathogen.

Introduction

During the past 30 years there have been attempts, in many countries, to forecast the date when potato blight (*Phytophthora infestans* (Mont.) de Bary) would become epidemic. The methods used merely specify weather thought to allow the fungus to sporulate and infect suitable hosts. The fact that some methods have succeeded is not only a tribute to their inventors but also indicates that the fungus is widespread in small amounts some time before the epidemic becomes obvious. Disease outbreak requires the presence of susceptible hosts, a source of the pathogen and weather favouring infection.

In England, the disease occurs first on potatoes, and although it later develops on tomato and a few other hosts, there is no evidence that these start epidemics. It has often been demonstrated that potato plants can be infected at any age when

provided with suitable conditions. However, the infrequency of attack on young crops in many potato-growing areas led to the belief that susceptibility increased with age. De Bruyn (1926) claimed that old plants were more susceptible because early plantings of six varieties were attacked and destroyed before later ones. The evidence was inconclusive as the mid-point of the epidemic was often reached earlier in the life of late-planted crops and the time taken for the foliage to be destroyed was often shorter. Müller also believed that susceptibility increased with age, but has recently found that *in vitro* ageing leaves become less susceptible to blight (Müller, 1953). Grainger (1956) claimed that there are two periods of high susceptibility, one early and one late in the growth of the plants. The possibility that the susceptibility of potato plants to *P. infestans* varies with changes in host physiology is important and worthy of critical examination. Apart from the task of producing an accurate and comprehensive definition of 'susceptibility', there are important practical difficulties in such a study. Attempts to assess ontogenetic changes of susceptibility from records of the progress of the disease on observation plots have failed (Beaumont, 1934; Limasset & Godard, 1941), probably because the rate of destruction seems to depend so much on the initial supply of inoculum and on meteorological conditions as well as the innate susceptibility of the host. Furthermore, potatoes grown under glass probably differ morphologically from field plants.

At present there seems to be no sure way of separating the influence of host physiology, source of the pathogen and weather on the development of epidemics. Until suitable experimental methods are devised we must rely on subjective interpretation of observations. This paper describes meteorological measurements throughout the growing period of the crop. The accompanying paper (Hirst & Stedman, 1960) emphasizes the frequency with which *P. infestans* overwinters in potato tubers, the first appearance of lesions above ground and the time and position at which subsequent ones develop.

Growers have always associated the appearance of potato blight with wet weather. In England, as in many other countries (Cox & Large, 1960), they know, to within approximately a month, when to expect the start of epidemics. Despite great progress in developing forecasting rules, this underlying constancy of the 'blight season' remains unexplained; the exact infection requirements of the fungus in crops are also unknown. Some methods of forecasting were developed empirically, others relied on 'models' of infection requirement based on the behaviour of the fungus in unchanging environments. In practical disease forecasting the derivation of the method is unimportant so long as it works. Many forecasters, however, would admit that success at present depends not only on the application of rules but also on judgement based on long experience of local crops and weather, and they would welcome additional guidance or improved rules.

The observations described below were made in the years 1951–8, while comparing the occurrence within and above crops of weather satisfying the forecasting rules by Beaumont (1947) (see Hirst & Stedman, 1956) and in testing how differences in the weather within crops differed from those above them. Earlier studies

of the weather within potato crops, their ecoclimate (Broadbent, 1950), were usually made at particular stages of growth and so did not reveal how the plants modify their environment as they grow (Waggoner, 1950; Waggoner & Shaw, 1951; Johannes, 1953). We think that the growth of crops produces a series of changes of great importance in the phenology of potato blight and that understanding them will help to improve potato blight forecasts.

Meteorological instruments and records

The effect of a potato crop on its immediate environment can properly be measured only by the difference between instruments within a crop and others similarly placed close to bare ridged ground. However, economy generally dictates that forecasting schemes rely on existing climatological facilities to avoid the cost of a network of special stations measuring conditions within crops. Therefore, although we studied the differences of conditions within the crop both from those close above bare ground and above crops at the standard height for climatological observation, we shall discuss only the latter in detail. It will be convenient in this paper to use 'weather' as a general term for meteorological conditions at any height, 'ecoclimate' (crop) for those within the crop and 'climate' (screen) for conditions prevailing within a standard pattern louvred screen 4 ft. above ground level.

In attempts to compare the effect of climate and ecoclimate on the activity of foliage pathogens it is essential to use continuously recording instruments, for the pattern of diurnal changes is even more important than precise measurement or more rapid fluctuations. Special instruments were necessary for measuring temperature and humidity, and distant reading mercury-in-steel wet- and dry-bulb thermographs were used. Though not ideally suited for microclimatology these are simple, reliable and robust enough to be operated for long periods in crops. Their disadvantages are that they respond slowly to temperature change because of the large heat capacity of their sensitive elements and they cannot be aspirated effectively in crops without drawing air from different levels in and above the foliage.

The recorders of these thermographs were housed in weather-proof shelters from which the capillary tubes led to the sensitive elements either in louvred screens 4 ft. above ground level or into the crop. The sensitive elements of the crop instruments were placed mid-way between two potato ridges and level with their crests, 8–10 in. above the base of the intervening furrow (Pl. 4). They were protected from sun and rain by well-ventilated covers, in 1951 these were of asbestos cement sheeting but as night minimum temperatures beneath them were slightly too high they were replaced in later years by covers of similar design made of white-painted weather-proof plywood.

Records are presented from the two stations which had instruments measuring both screen and crop temperature and humidity. At Rothamsted temperature and relative humidity were calculated at every even hour in the growing seasons of 1951–5. In subsequent years and for the records from the Experimental Husbandry Farm, Terrington St Clement, Kings Lynn, Norfolk, only periods with relative

humidity not less than 90% were measured, using the transparent plastic grid described by Hirst & Stedman (1956). Rainfall, sunshine and wind records were obtained from standard climatological instruments at each station. All times referred to are Greenwich Mean Time.

Crop and disease records

At Rothamsted a quarter-acre plot was planted each year for observations of weather and spread of the fungus. At Terrington the screen observations were made in the meteorological enclosure and crop instruments were placed in unsprayed plots of the variety Majestic (King Edward in 1955) in a blight control experiment (see Large, Taylor, Storey & Yule, 1954).

Cultivation and manuring followed local practice. At Rothamsted, for example, tubers were 15–18 in. apart in rows 27–28 in. apart; the usual manuring was 10 tons of dung per acre and 5 cwt./acre of a compound fertilizer (7% N, 7% P_2O_5, 10½% K_2O), and when dung was omitted fertilizer was applied at 15 cwt./acre. The height of the stations above mean sea level and their soil types are as follows: Rothamsted, 450 ft., clay with flints; Terrington, 13 ft., heavy silt. Some indication of the development of the crops at Rothamsted is given by the dates when the following foliage-density categories were reached (see Text-figs. 3, 4).

½ Half the plants emerged above ground.

1 Plants easily distinguishable from a distance as individuals. Foliage meeting neither in the rows nor across them.

2 Foliage meeting in but not across the rows, stems erect.

3 Foliage meeting both in and across the rows and forming a complete canopy, stems erect.

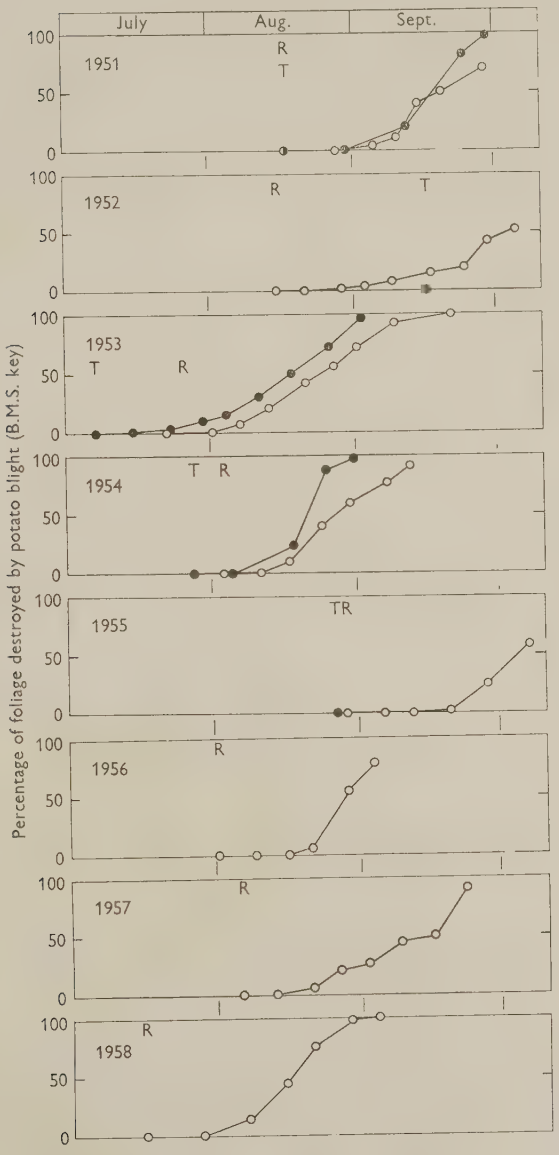
P Indicates prostrate stems with erect side branches and is combined as 2P or 3P to indicate both the total amount and the state of the foliage.

R Date of blight 'outbreak' in the region.

The disease records in Text-fig. 1 show the regional outbreak dates of the disease at Rothamsted (R) or Terrington (T) and its progress on unsprayed crops of 'Majestic', at both stations. The British Mycological Society's Key (Anon., 1947) was used to estimate the amount of foliage destroyed by blight; an outbreak was defined according to Large's (1953) convention, as the first record of the disease having destroyed 0·1% of the foliage. At Terrington, the disease records were made in the same crops as the meteorological observations. At Rothamsted the records of outbreak and progress in Text-fig. 1 refer not to the ecoclimate observation plot but to unsprayed plots on blight control experiments elsewhere on the same farm.

Diurnal periodicity of temperature and humidity in crop and screen at Rothamsted, 1951

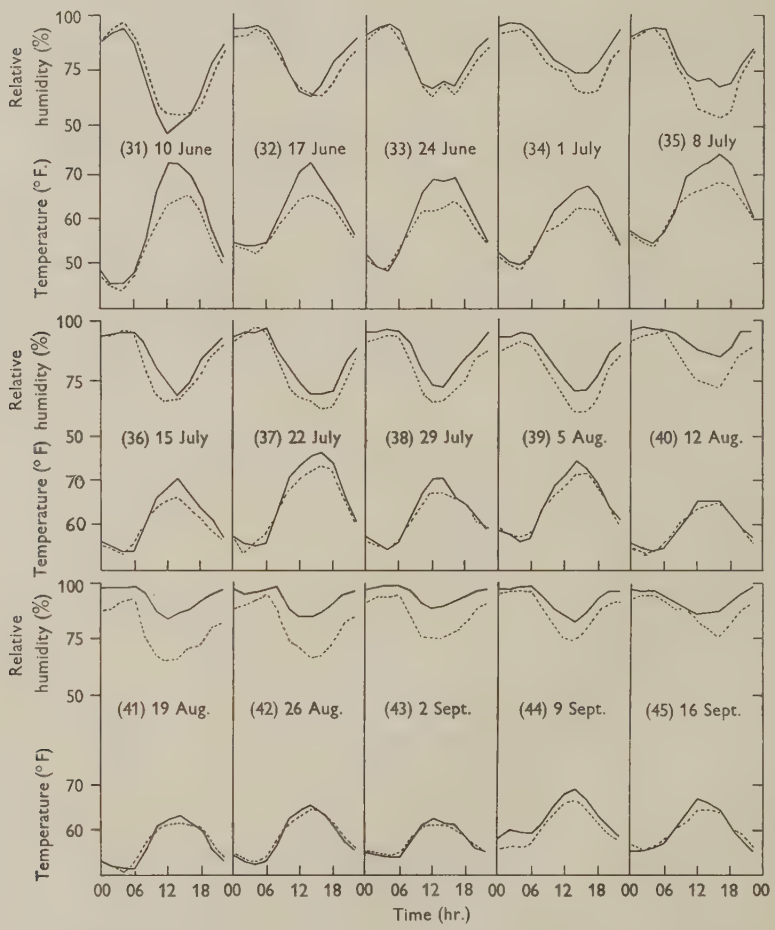
An effective comparison between climate and ecoclimate must show differences at all times, at all stages of crop development and with all types of weather. The most practical method of presentation is to calculate average diurnal periodicity curves



Text-fig. 1. Dates of outbreak of potato blight at Rothamsted (R) and Terrington St Clement (T) and progress of the disease measured by estimates of percentage foliage destroyed (Rothamsted, open circles; and Terrington, black circles).

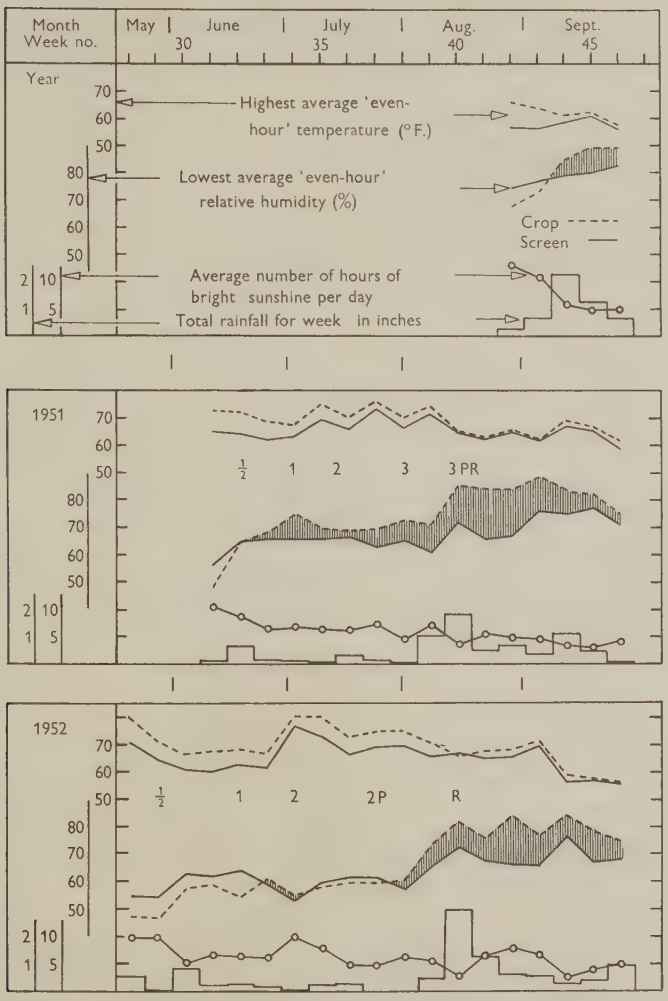
during successive weeks,* although this splits wet and dry spells arbitrarily. Calculating the periodicity during overlapping periods as in running means would be better but is a laborious refinement and was not adopted.

Full details are given of the diurnal periodicities of temperature and humidity during the growing season of 1951 (Text-fig. 2), a year which demonstrates clearly



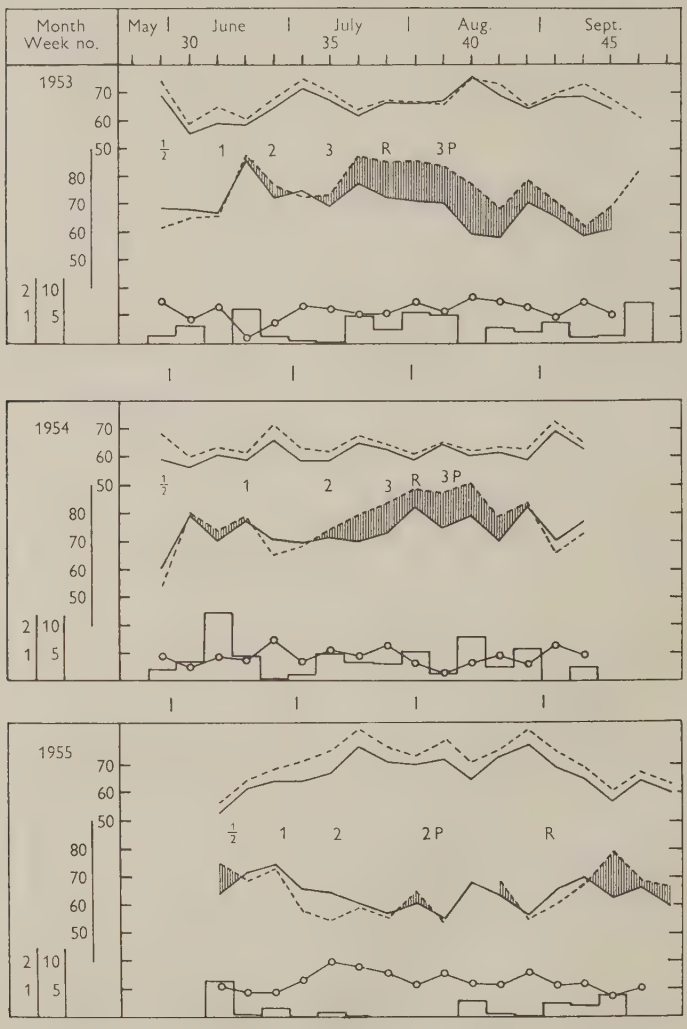
Text-fig. 2. Diurnal periodicity of air temperature and relative humidity at Rothamsted in 1951. Weekly averages at each 'even' hour joined by dotted lines for records made in a louvred screen 4 ft. above ground or by solid lines for those made low among the foliage of a potato crop. The 'Shaw number' of each week (Shaw, 1933) and date of its last day are shown in each panel.

* Week numbers are as used in the Meteorological Office Crop Weather Scheme and start from 6 November (see Shaw, 1933).



Text-figs. 3, 4. Changes in the relationship of climate and ecoclimate of potato crops at Rothamsted, 1951–5. Data are abstracted from diurnal periodicity curves for successive weeks similar to those shown in Text-fig. 2. Curves join the highest 'even-hour' temperatures and lowest 'even-hour' relative humidities for records from crop (dotted line) and screen (solid line) instruments, vertical shading indicates where lowest crop humidity exceeds that in the screen. Curve with open circles joins weekly average sunshine (hours per day) and histogram shows total rain for week. Symbols (½, 1, 2, 3, P) indicate state of crop according to key described in text and (R) date of blight outbreak. Dates by month are approximate and Shaw numbers are plotted at the last day of the week.

the succession of changes repeated in subsequent years (for details see Hirst, 1955*b*). In 1951, planting (17 May) was delayed by an exceptionally cold wet spring and by week 31 (Text-figs. 2, 3) fewer than half the plants had emerged. At this



Text-fig. 4 (for legend see Text-fig. 3).

time the crop had little influence on its environment; the ecoclimate was similar to conditions at the same height above bare ridged ground but differed appreciably from those in the screen. Week 31 was dry and sunny, at noon the air close to the

soil averaged 10° F. warmer than in the screen but at night there was little difference. The higher temperatures close to the ground at midday resulted in the air there, although of a higher dew-point, having a relative humidity about 5% lower than in the screen.

Plants met along the rows in week 35 and across them in week 38. While this canopy of foliage was developing the weather was warm and sunny with occasional showers but no heavy rain. Conditions within the crops became progressively less like those above bare soil, the difference between midday temperature in crop and screen declined and relative humidity was higher in the crop at all times of day. These changes would be expected to accompany the formation at the top of the crop of an 'outer active surface' (Geiger, 1950), which assumed many of the former characters of the air-soil boundary, and beneath which a shallow stratum of air of higher dew-point developed within the crop as a result of active transpiration and decreased ventilation.

After being thoroughly wetted by heavy rain at the end of week 39 and in week 40, the soil only partly dried. Air temperature at the two heights became almost equal, had a smaller diurnal range and a lower average than before the rain. The most notable change was in relative humidity, which rose less in the screen than in the crop, where it averaged over 90% for more than 16 hr. per day. This relationship between weather in and above the crop persisted for 5 weeks. Blight outbreak occurred in week 40 and by defoliating the crop it became an important factor in determining the ecoclimate. More than half the foliage was destroyed by the end of week 44 and soil again became exposed. The relationship between the diurnal periodicity curves from crop and screen instruments tended to return to that shown in June. A complete reversion would not be expected because of the shorter days and lower temperatures during September.

In 1951 and each of the subsequent years, the diurnal periodicity of temperature and relative humidity in crop and screen showed a similar succession of relationships, although it was influenced by the weather. When there was considerable bare ground exposed, the air in the crop at midday was warmer and less humid than at 4 ft. above ground, particularly when the weather was dry and sunny. The growth of the foliage and the amount of sunshine and rain determined how soon this pattern changed to one where the temperature differences were much smaller, but the relative humidity in the crop remained higher at all times of day. When the weather during the early stages of the growth of the crop was dull and wet, the differences in temperature were smaller and the crop level humidity was increased, but only slightly. It was not until a dense foliage canopy had formed that rain had its most pronounced and lasting effects. These were almost complete elimination of the temperature differences between the crop and screen instruments and the persistence of relative humidities over 90% among the plants for many hours each day. Eventually the death of the crop, whatever the cause, re-exposed the soil and the relationship partially reverted to that found early in the season.

Weather changes associated with blight outbreak

The curves for 1951 (Text-fig. 2) show that changes in temperature and relative humidity likely to be important pathologically may be inferred either from the peaks of the diurnal temperature curves and the lowest points on the diurnal humidity curves, or by a measure of the proportion of the curves above or below some level believed to be significant biologically. Therefore the highest of the even-hour average temperatures and the lowest of the corresponding relative humidity values from each instrument at Rothamsted were plotted for successive weeks in Text-figs. 3 and 4, for the years 1951–5. The same figures also show, week by week, the total rain and the average number of hours of sunshine per day. In each panel, symbols denote the density of the crop according to the scale mentioned above and the date of regional blight outbreak. Periods when the lowest crop humidity is greater than that in the screen are shown by vertical shading of the area between the two curves. Sporulation of *P. infestans* (Crosier, 1934) and the persistence of water droplets on foliage depend on high relative humidity, so the length of periods when it was not less than 90% are plotted in Text-figs. 5 and 6. The curves for screen and crop instruments join points, 3 days apart, representing the mean number of hours per day, during the preceding and following 3 days, when relative humidity was 90% or more.

The temperature and humidity at Rothamsted in 1951 have been fully described, but the year is included in Text-fig. 3 for comparison with later years and to show how the relationships between climate and ecoclimate (Text-fig. 2) were influenced by sunshine and rain. The duration of relative humidity not less than 90% (hereafter called 'high humidity') as represented by the 6-day running means in Text-figs. 5 and 6 varied in the screens between 4 and 16 hr. per day; in the crops the range was from 8 to 21 hr. In June and July 1951, high humidity lasted only a little longer in the crop than in the screen. At both Rothamsted and Terrington the rain in the first week of August (week 40) caused the crop and screen curves to diverge and blight outbreaks soon followed. Throughout the season wet spells cause transient peaks in the curves of the duration of high humidity in screens. In crops with dense foliage the effect of wet spells persists because the foliage retards the drying of the soil surface. The similarity of duration of high humidity in crops at the two stations (Text-fig. 6) is paralleled by very similar progress of the disease (Text-fig. 1).

In 1952, the crop at Rothamsted was planted earlier (29 April) than in 1951 and half the plants had emerged by 23 May (week 29). The summer was dry and by mid-July the plants were suffering from drought and had become prostrate. Consequently at no time was a complete canopy formed and the midday relative humidity at crop level (Text-fig. 3) was usually less than in the screen until late July (week 37). Dull wet weather again came in early August and blight appeared in the middle of the month, but developed more slowly than in 1951, probably because of the poor development of the haulm (Text-fig. 1). At Terrington blight was not found until mid-September and then only on a few leaves in the experi-

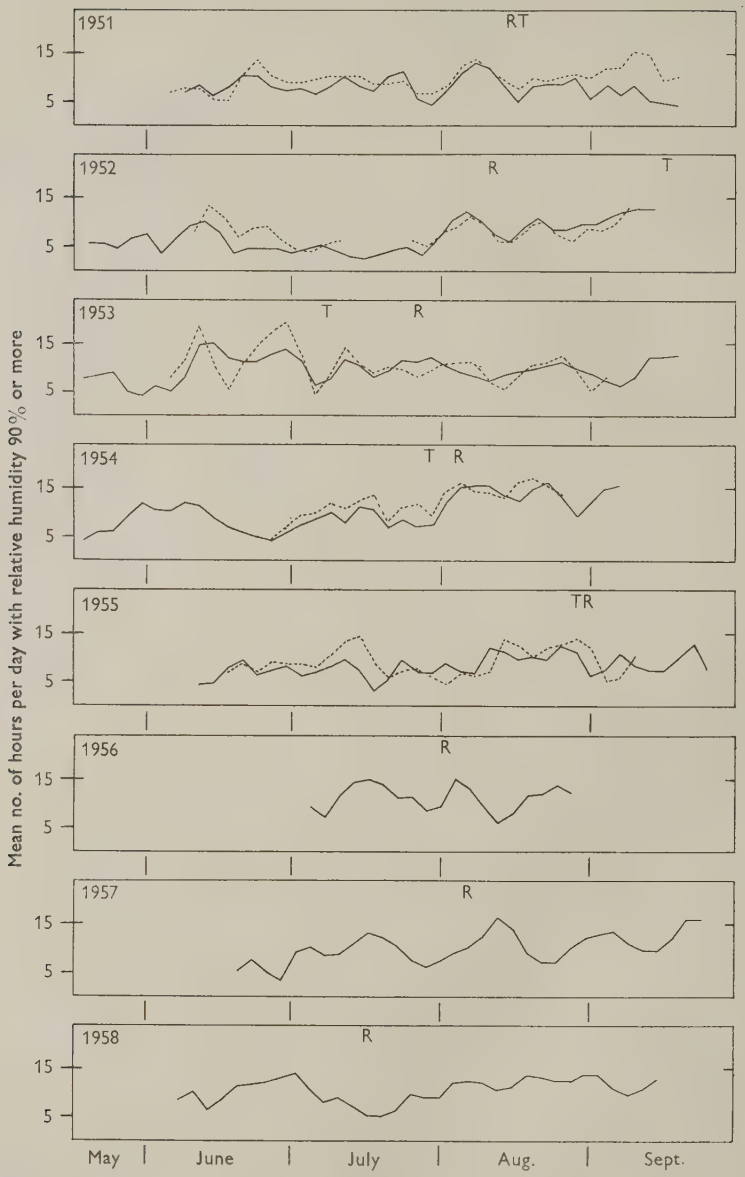
mental crop. In the screen high humidity lasted as long at Terrington as at Rothamsted, but in the crop it was less persistent and the Terrington curve never reached 16 hr. per day (Text-figs. 5, 6).

In 1953 potatoes were planted at Rothamsted on 14 April. The crop grew slowly until late May, but then quickly so that a complete foliage canopy had formed early in July. The bare ground characteristics of the crop record disappeared earlier than in 1952 and high humidity was common in the crop from June until August (Text-fig. 6). Blight appeared early, particularly at Terrington, but drier weather from mid-August onward slowed the rate at which the haulm was destroyed.

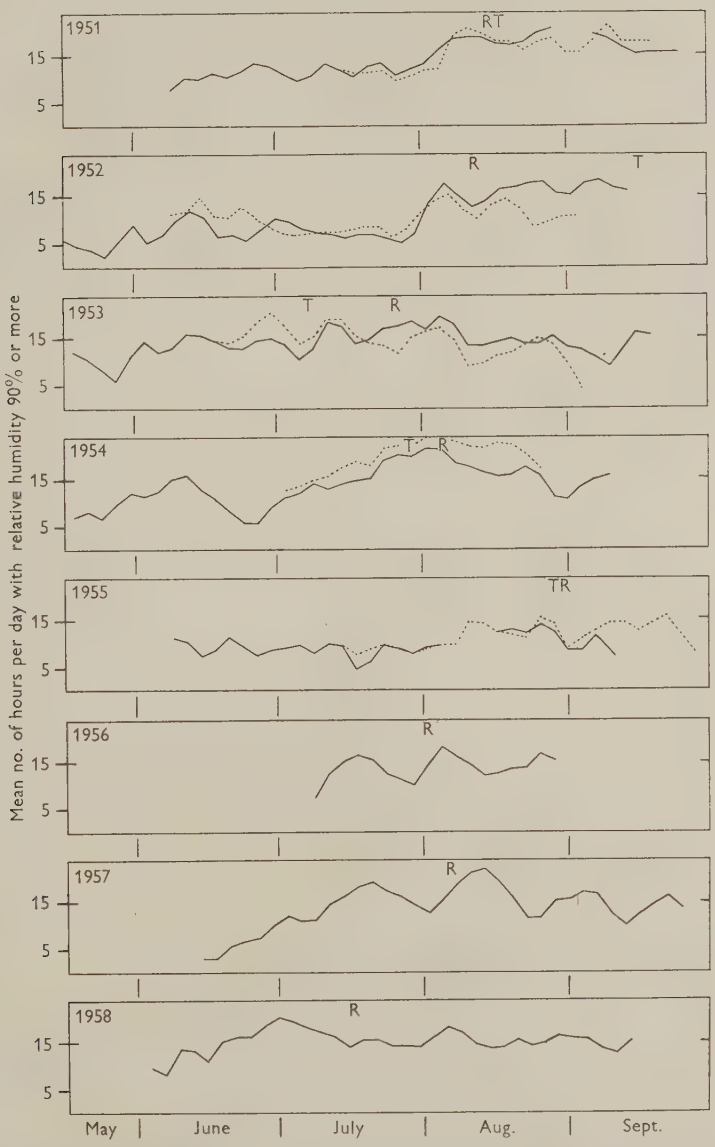
In many ways 1954 resembled 1953. Half of the tubers planted on 13 April had produced plants by 26 May. Except for a short dry spell in late June, this month and July were cool and dull with more rain than the average. Until September when blight had destroyed most of the foliage, the lowest point of relative humidity curves in crop was less than in the screen during 3 weeks only (Text-fig. 4) and there were several occasions when the fungus spread around initial sources (Hirst, 1955*a*). The instruments at Terrington were installed in the second half of June, but the brief records bore the same relationship to blight outbreak as at Rothamsted.

In 1955, only half the tubers planted at Rothamsted on 30 April had produced plants by 8 June. Growth was further delayed, at first by low temperature and then by lack of rain. As a result not all the soil was covered and there was little possibility of a persistent blight-favouring ecoclimate developing even in late July or early August. The disease outbreak was not until the end of August and then it developed very slowly. Weather likely to encourage the spread of blight occurred during two periods, first in week 40, when the crop instrument was unfortunately defective, and second in weeks 44 and 45. Curves for the persistence of high humidity at Rothamsted differed greatly from those in the two preceding years and, where complete, indicated that blight would be scarce and late. Terrington, like much of the east coast, had persistent fogs in July but there was no rain and most crops remained free from blight until the end of August and even then it was rare.

After 1955 the persistence of high humidity was recorded only at Rothamsted. In 1956 the records are brief because late cultivations delayed installing the instruments and the epidemic was severe and short. The unusually small difference between the peaks of high humidity curves (Text-figs. 5, 6) is notable and probably resulted from dull spells with unusually heavy rain (see Hirst & Stedman, 1960, fig. 10). In 1957 June was very sunny with less rain than usual, but wet weather came in mid-July. As a result the crop grew well and within it periods of high humidity, which had been unusually brief in June, rose above 15 hr. per day by mid-July. During August, dry spells slowed the development of the disease after its outbreak at the beginning of the month. Persistent high humidity developed very early in crops in 1958, only 1953 was comparable, and it is noteworthy that the earliest outbreaks occurred in these years.



Text-fig. 5. Curves of the duration of high relative humidity in louvred screen 4 ft. above ground at Rothamsted (solid lines) and Terrington St Clement (dotted lines). Curves join points three days apart each of which represents the mean number of hours when relative humidity was 90 % or more on the preceding and following three days. Blight outbreak dates at the two stations are indicated by (R) or (T) respectively.



Text-fig. 6. As for Text-fig. 5, but the records of instruments set low among potato crops.

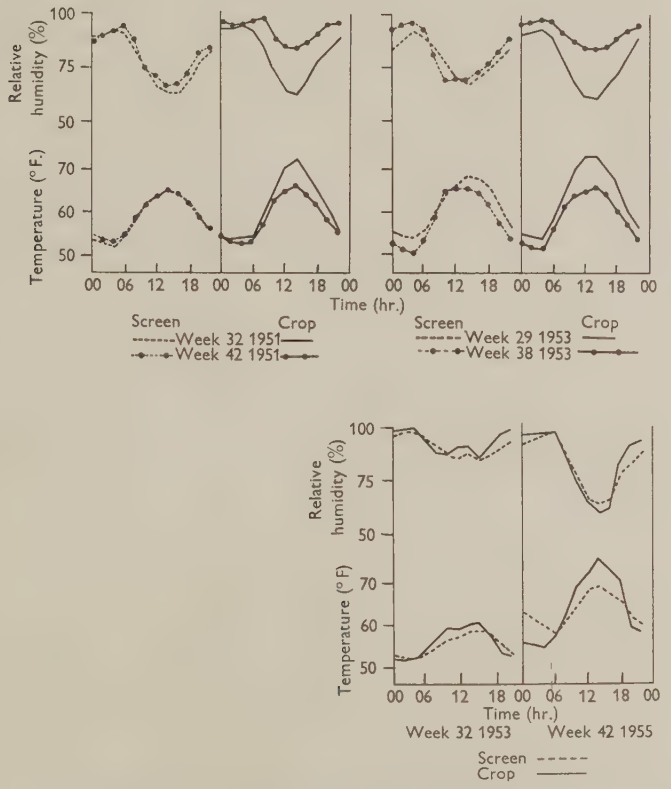
Discussion

In most of southern England (Large, 1958) blight outbreaks are rare before mid-July, but, except in the driest years, always occur by mid-August. Such regularity could result from a change in climate with time, as no doubt it does in countries with seasonal rains. There is little evidence to suggest any such change in the climate of the British Isles (Hirst, 1955*b*). Bourke (1953, 1955) showed the importance of certain weather systems to potato blight forecasting, but these do not explain the regularity of blight outbreak because they are by no means restricted to the blight season and each lasts only for a few days at most. Although there are changes favouring blight in long-period weekly weather means at Rothamsted between mid-July and mid-August, they too seem inadequate to account for its regular appearance. They amount (Hirst & Stedman, 1960, fig. 10) to about $\frac{1}{10}$ in. more rain each week, 1 hr. less sunshine per day and a fall in temperature of 1–2° F. Climatic averages are renowned for concealing important factors but can scarcely be blamed for not revealing large changes in weather peculiar to potato crops.

Our observations have been presented so as to emphasize changes in ecoclimate regularly associated with particular stages of growth rather than to suggest immediate detailed improvements in methods of forecasting. They show that the potato crop modifies its environment as it grows and that the ecoclimate at any time depends on both recent and present weather above the crop. Early in growth, while much bare ground is exposed, the diurnal fluctuations of temperature and relative humidity are larger at crop level than in the screen and, particularly at midday in dry weather, the air near the ground may be both warmer and less humid. At this stage of growth opportunities for infection occur only while wet spells dominate climate and ecoclimate; these spells are usually brief, so although the fungus may spread, it cannot multiply enough to become epidemic. When a dense canopy of foliage has developed, humidities near saturation, particularly after heavy rain, persist much longer within the crop than above it, although the temperatures remain very similar. Such conditions have been found typical of potato blight epidemics.

The way density of foliage influences ecoclimate can be further illustrated by selecting weeks with similar climate, one early and the other late in growth, and comparing their ecoclimates. In 1951 emergence was just completed by week 32 (ending 10 June, Text-fig. 7), when there was an average of 8·8 hr. of sun per day, a total of 0·65 in. of rain; by week 42 (ending 26 August) which had 4·9 hr. sun per day and 0·70 in. of rain there was a somewhat straggled full canopy of foliage and blight had appeared. The second pair, from 1953 (week 29, ending 27 May, and week 38, ending 29 July) represent the same stages of growth and blight attack and had respectively 7·4 and 5·3 hr. of sun per day and totals of 0·24 and 0·52 in. of rain. In the earlier week of each pair the range of temperature and humidity is greater in the crop than in the screen, the difference being greatest in the daytime. With dense foliage, similar weather above the crop in the second weeks caused little difference in temperatures, but crop humidities were continuously higher than in

the screen, particularly in the daytime. Examples already given show that differences in density of foliage can cause important changes in ecoclimate. Their action is always one of modification within limits imposed by climate and is often much less pronounced than in the weeks represented in the upper part of Text-fig. 7. Thus, before individual plants have met across the rows the crop has a much



Text-fig. 7. Diurnal periodicity of air temperature and relative humidity, in crops and screens at Rothamsted, in weeks selected for reasons described in the text.

smaller effect on ecoclimate humidity during wet spells. During week 32 (ending 17 June 1953) there was 0·9 hr. of sunshine per day only and 1·23 in. of rain (Text-fig. 7). Temperatures were low and varied little in both crop and screen (as in wet weather at any stage of growth) but the relative humidity curves were much more nearly coincident than would be expected under dense foliage, in a similarly wet week later in the season. The foliage present in week 32 was not dense enough to retain an ecoclimate sufficiently humid for blight outbreaks to

result, whereas later in the season the disease developed during weeks with much more sun and less rain. In hot dry years when growth is restricted a full canopy may never develop. Then records typical of the emergence period may persist for months because dry soil is still exposed. This happened in 1955 (Text-fig. 4) and shows clearly in the diurnal periodicity curves for week 42 (ending 3 September, Text-fig. 7) when there was 5·5 hr. of sunshine per day and no rain.

In our experience, rapid blight attacks have always been associated with much more persistent high humidity within the crop than above it. This relationship has only developed with dense foliage and wet weather, and is evidence of the importance of ecoclimate in governing the outbreak and development of potato blight. In the early part of the season the duration of high humidity in screen (Text-fig. 5) and crop (Text-fig. 6) moved in unison. Later, although in the screen the curves continued to rise and fall with the passage of wet spells, in crops with dense ground cover over wet soil, high humidity persisted for a large part of the day even when the weather above had become dry. This change, which is often represented in the crop curve by an obvious discontinuity lacking in the screen, provides long and frequent opportunities for *P. infestans* to infect. Its presence and relation to blight outbreak will be more clearly seen when a ruler is laid across the lower parts of Text-figs. 5 and 6 up to the 15 hr. marks. Unlike the screen curves which rarely and briefly appear above these marks, the curves from crop instruments exceed 15 hr./day for considerable periods just before and during rapid epidemics. The date when crop curves cross and remain above 15 hr./day for at least three points on the curve (a total of 12 successive days) seems to be a good indication that an epidemic will follow. There is some evidence that the earlier the indication the longer the interval between it and blight outbreak, but it is usually 1–3 weeks. Slight attacks as in 1952 and 1955, or sporadic infections early in the season (Hirst, 1955*a*), may occur with less persistent high humidity. Once the disease has appeared there is a relation between the excess of the crop curve over 15 hr./day and the rate at which the first half of the foliage is destroyed (Text-fig. 1). After this stage, the soil is increasingly exposed so that the ecoclimate is modified.

There would be little purpose in giving yet another demonstration that potato blight flourishes in wet weather even if it supported recent improvements in forecasting such as the incorporation of high humidity requirements in forecast criteria (Bourke, 1953, 1955; Smith, 1956). But, to understand the role of ecoclimate in the epidemiology of potato blight is worthwhile. This problem is far from simple and at present objective measurement of the ecoclimate's importance in isolation from other factors seems impossible. The ecoclimate probably affects the frequency of initial sources of the fungus and the number of spores which are produced, dispersed and able to cause new infections. Its changes are associated with variations in weather and the size of individual plants, and therefore with the extent and distribution of susceptible targets and possibly with physiologically induced changes in susceptibility as plants age. The difficulties of designing experiments able to eliminate the effects of variable inoculum and plant size on crops of a variety of ages, mean that the immediate aim must be to suggest reasons

for the possible effect of ecoclimate on potato blight phenology, and to enable its importance to be judged on the fullest possible circumstantial evidence.

The activity of *P. infestans* during the growing season of potato crops is divided almost equally by the date of blight outbreak into early, cryptic, and later epidemic phases (Hirst, 1955*a*; Hirst & Stedman, 1960). In our experience, blight outbreak ranged over little more than a month and was not accompanied by lasting changes in climate thought sufficient to account for such a dramatic change in the multiplication of the fungus. Changes in ecoclimate, such as those described, offer one possible explanation. During the early phases of growth when soil is exposed and ventilation is little impeded, day temperature and humidity are often less favourable to *P. infestans* among crops than above them, although at night they are similar at either height. When soil beneath a dense canopy is wetted, ecoclimates develop which are followed by blight outbreaks. This change ensures frequent occurrence of long humid periods which persist within the crop despite dry weather above. These conditions allow abundant sporulation and the persistence of water deposits on foliage ensures a high percentage of successful infections. By contrast, during the earlier stages of growth spores would be formed, and water deposits would persist, only during relatively brief wet spells when climate dominated ecoclimate. The theory therefore supposes that the growth of potato crops is often self-destructive for they progressively modify their environment to favour one of their most serious pathogens.

We wish to thank the Farm Director and staff of Terrington Experimental Husbandry Farm for the facilities and assistance they provided. Also, we are grateful to the Director General of the Meteorological Office for the loan of instruments.

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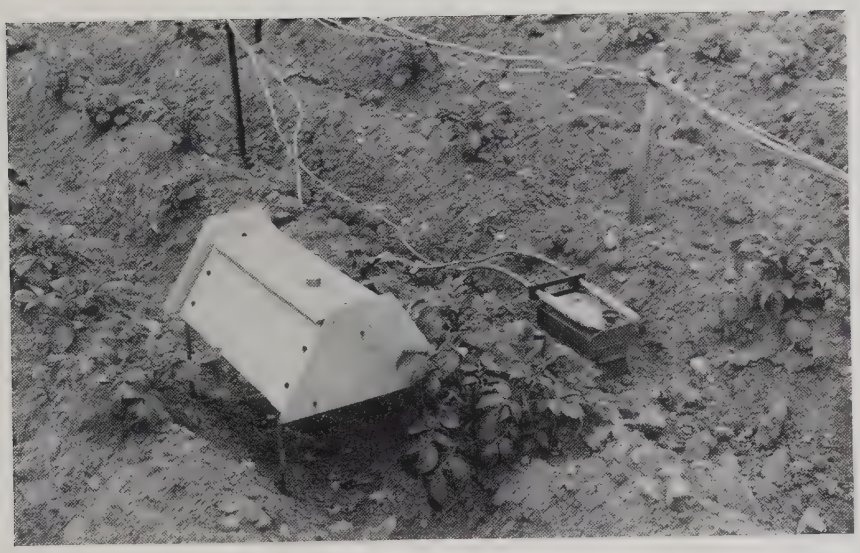
EXPLANATION OF PLATE 4

Sensitive elements of a wet- and dry-bulb mercury-in-steel thermograph in position in a young potato crop. Cover temporarily displaced one row.

(*Received 2 December* 1959)



HIRST AND STEDMAN—*The epidemiology of* Phytophthora infestans. *I*

(*Facing p.* 488)

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THE EPIDEMIOLOGY OF *PHYTOPHTHORA INFESTANS*

II. THE SOURCE OF INOCULUM

By J. M. HIRST and O. J. STEDMAN

Rothamsted Experimental Station, Harpenden, Herts

(With 13 Text-figures)

Of 3260 blighted tubers planted as seed in 5 successive years (1954–8), twenty-one produced stems invaded from below ground by *Phytophthora infestans* (0·79 and 0·52 % respectively of those infected artificially and naturally). Only in 1955 was no stem found to be invaded, in every other year they started epidemics in experimental plots. On average the first stem lesions were found 62 days after planting and 38 days before blight outbreak became general in the district.

There was evidence of two patterns of dispersal for *P. infestans*. Distant spread was probably by air-borne sporangia, but that near to the initial sources probably resulted from sporangia transported in water. The effects of initial sources of infection on the date when the disease became general could not be traced for more than a few hundred yards. Blight often reached crops remote from the experiments at the same time as it infected every plant within them; this often happened during the weather which elicited disease forecasts.

Under certain conditions of soil moisture, *P. infestans* from artificially infected tubers planted as seed seemed able to pass through the soil to infect leaves near it and to start epidemics without invading stems from below ground. Attacks originating in this way developed similarly to those started by invaded stems, but their importance and the conditions limiting their occurrence have yet to be determined.

Introduction

Hirst & Stedman (1960) described how the date of potato blight 'outbreak', as defined by Large (1953), was related to changes in the weather above and within crops. Such observations could not establish that weather controlled the phenology of the disease because host susceptibility or the supply of inoculum may have differed between years or between different months within years. We now discuss the supply of inoculum and describe further experiments on the origin and development of semi-natural epidemics in small potato plots at Rothamsted (Hirst, 1955*a*).

How *Phytophthora infestans* (Mont.) de Bary overwinters has long been one of the classic problems of mycology. Since the subject was summarized by van der Zaag (1956), it has been discovered that oospores are formed abundantly between isolates of two mating groups, one of which seems to occur only in Mexico. Although the oospores may not be important in Europe, it would be wise to renew the search for them in cross-matings because of their potential importance both in the production of new physiologic races and the origin of epidemics. Tests of oospore germination (Smoot, Gough, Lamey, Eichenmuller & Gallegly, 1958), and their distribution, so far suggest that few would succeed in infecting foliage but it would be foolish to ignore them, for exceptional infections could develop into epidemics.

Although in New Zealand *P. infestans* may overwinter on wild perennial *Solanum* spp. (Driver, 1957), there is still no evidence that this happens in England on any host other than potato. Since 1950 the fungus has been isolated, in Harpenden, from *Petunia hybrida* and *Datura stramonium* (Hirst & Moore, 1957), once from shaded leaves of *Solanum nigrum* and in five consecutive years from senile shaded leaves of *S. dulcamara*. On the last, which is a perennial, there was no sign of systemic infection; lesions appeared at a late stage of epidemics and affected leaves soon fell.

It has long been known that *P. infestans* can overwinter in potato tubers and occasionally succeeds in growing up stems to produce lesions above ground. Invaded stems are usually weak and are killed when girdled by the fungus, so it is not surprising how rarely they can be found among the multitude of healthy stems from over 12,000 tubers planted in each acre. Most attempts have failed to overcome the dilemma described by Brooks (1919) that, by the time a group of plants more heavily infected than the rest of the crop is noticed, it is usually too late to decide how the infection originated. To avoid this difficulty the invaded stems must be found before other plants are infected, then the proximity of later infections will be strong evidence that the invaded stems initiated the attack. This has been achieved by planting a high proportion of diseased tubers in plots small enough for every plant to be examined carefully each week. Even so, there was no possibility of proving mycelial continuity between the above ground lesion and the diseased tuber, for this would involve removing the plant and thereby prejudicing the future development of the attack. The compromise we adopted was to trace lesions as far below ground as possible without unduly disturbing the root system and removing a leaf when its petiole became invaded, so that the presence of *P. infestans* could be confirmed by the production of sporangia.

The origin of attacks from blighted seed tubers

From 1955 to 1959 plots up to $\frac{1}{4}$ acre were planted with healthy and diseased tubers in patterns resembling that used in 1954 (Hirst, 1955*a*). A frame of healthy tubers surrounded an area where rows of diseased or healthy tubers alternated. Healthy seed was necessary to ensure that a reasonably dense crop developed despite the failure of some diseased tubers to produce plants and also to indicate how the fungus spread. Tubers naturally infected the preceding summer were often much shrunken at planting time and were usually invaded by organisms in addition to *P. infestans* but all had at least one live sprout; in some varieties the sprouts were often long. The artificially infected tubers looked more normal. Lesions were obvious and differed in size with variety and interval since infection but the tubers generally retained their shape. Equal numbers were infected at rose and heel end by inserting sporing mycelium from a culture on chick pea (*Cicer arietinum*) agar into a $\frac{1}{4}$ in.-deep wound which was sealed with molten paraffin wax. Healthy tubers were usually of the variety Majestic. In 1954 tubers were planted in flat ground but in later years they were hand-planted in the base of a ridge. Unless otherwise

stated, rows were 28 in. apart and all tubers were accurately planted at 2 link (approx. 16 in.) intervals within the row, so that the position of each tuber could be examined every week for emerged plants or infections on their stems or leaves. None of the plots had borne a crop of potatoes during the previous 4 years. To avoid damaging or burying weak shoots, weeding was done by hand, except in 1959.

We shall use 'focus' to mean: 'Site of local concentration of diseased plants or disease lesions, either about a primary [initial] source of infection or coinciding with an area originally favourable to establishment, and tending to influence the pattern of further transmission of the disease.' (Anon., 1953). The terms 'primary' and 'secondary' have become the subject of contradictory conventions; to avoid confusion they will not be used.

Experiments in 1955

A plot of rather more than $\frac{1}{4}$ acre was planted with 4004 tubers between 28 April and 9 May 1955. Within this 450 diseased tubers were planted (Table 4) in areas containing either naturally or artificially infected tubers (Fig. 1); rows of healthy tubers alternated with rows in which every other tuber was diseased. The healthy tubers were of the variety Majestic except where diseased Majestic tubers were interplanted with healthy Ulster Cromlech tubers so that they could be easily distinguished. No blight was found on foliage until 30 August, when one of the four stems of a plant derived from a healthy Majestic tuber (Fig. 1, row 7, plant 71) bore a single lesion about 4 in. long. Unlike the lesions typical of invaded stems which appear at the base and gradually extend upwards invading successive leaf petioles, this lesion was at the top of the stem like those commonly caused by spores dispersed during epidemics. No invaded stems were found in the plot at any time, and although the source of this first infection is unknown it seems probable that it came from outside the plot. Since 12 July, a series of potted potato plants with blight-infected foliage had been exposed, one each day, in a crop of Majestic about 150 yd. to the south. New infections were discovered close around the sites of infected plants exposed on 1 day in July and 6 in August, and spread was particularly intense on 13 August. There could be several reasons for failing to record any invaded stems in 1955. Although the experiment contained 450 diseased tubers and later years suggested this should suffice to produce at least one invaded stem, it was not enough to ensure this happening. Invaded stems might have been overlooked during the weekly examinations, but even so neighbouring plants should have been infected and noticed later. Hot dry weather, particularly the high midday temperatures at the boundary between air and dry soil, may have prevented the fungus emerging above ground or prevented infection before the stems and fungus died. Finally, the fungus may have died before the tubers were planted, although this seems improbable in view of the contemporary glasshouse experiment described below. The disease developed very slowly, by 20 September (Fig. 1) only 4% of the plants were infected, and most of these were outside the areas where diseased tubers were planted. The healthy guard rows were planted almost 2 weeks later than the areas containing blighted tubers so they withstood the drought in June

and July (Fig. 10) better, and offered more foliage for infection from mid-August onward when the older plants were dying. The pattern of infected plants on 20 September is therefore consistent with an original introduction from outside the plot followed by limited spread within it.

Subsamples, each of twenty-four artificially infected tubers, were taken from stocks of the four varieties in the field, potted on 3 May and then kept well watered in an unheated glasshouse. Weekly examinations revealed the first typical invaded

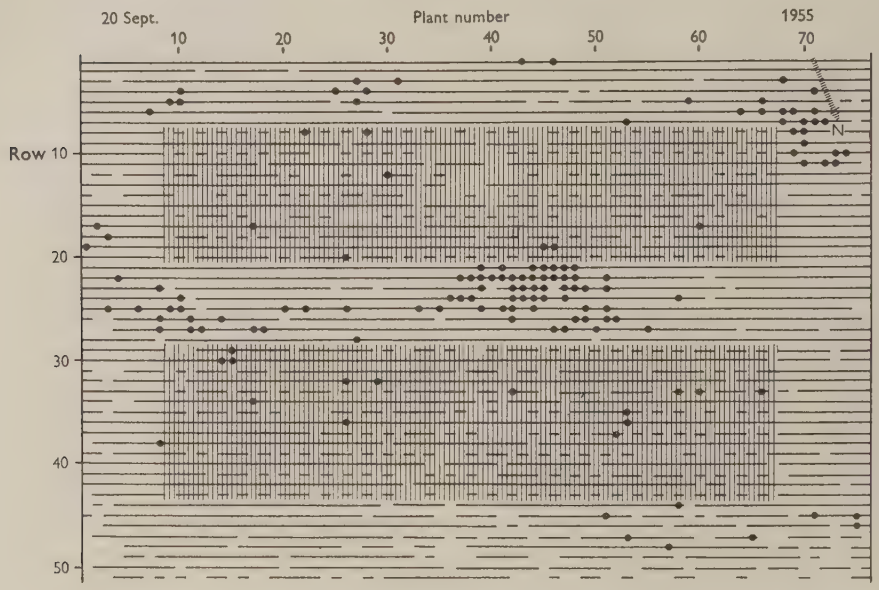
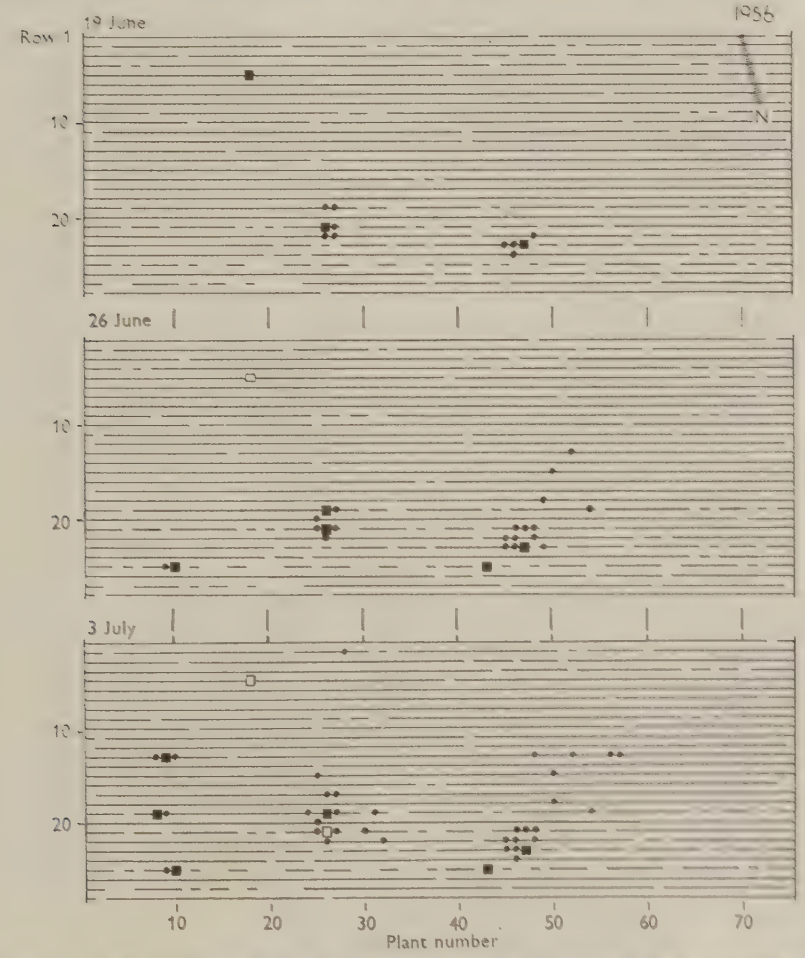


Fig. 1. Plan on 20 September 1955 of plot in which blighted tubers were planted showing the plants which had emerged (–) or where the foliage had become infected (●). Continuous black lines indicate that plants had developed from all tubers planted. Two areas, between plants 9–68 in rows 8–20 and 29–43 contained one blighted tuber in every four planted (see text), the northern area being planted with artificially infected tubers, the southern with tubers infected naturally. All other tubers healthy.

TABLE 1. *Proportion of artificially infected tubers which produced invaded stems when grown in an unheated glasshouse: May–July* 1955

Variety	Proportion with invaded stems	
	Plants	Stems
Majestic	1/24	1/70
King Edward	2/24	2/81
Ulster Cromlech	4/24	5/57
Ulster Chieftain	3/24	6/84
Uninfected Ulster Cromlech as test of aerial spread	0/12	0/48

stems on 26 May. By 6 July they had appeared from 10% of the tubers although only 4·8% of the total stems were invaded. All these lesions were typical of invasion from the tuber and no lesions developed on twelve Ulster Cromlech plants from healthy tubers distributed at random throughout the experiment as checks on infections resulting from spore dispersal. It must therefore be assumed that the



Figs. 2, 3. Plans of plot in which blighted tubers were planted in 1956 showing plants emerged (–), with invaded stems active (■) and inactive or dead (□) or with aerial blight lesions on foliage (●). Odd numbered rows (except row 1) planted with blighted tubers, even numbered rows with healthy tubers, also two healthy tubers at each end of all rows. Invaded stems were first found on 12 June before any aerial infections were seen.

fungus was alive in at least 10% of the tubers planted in the field and that lack of invaded stems was not because the fungus had died before the tubers were planted.

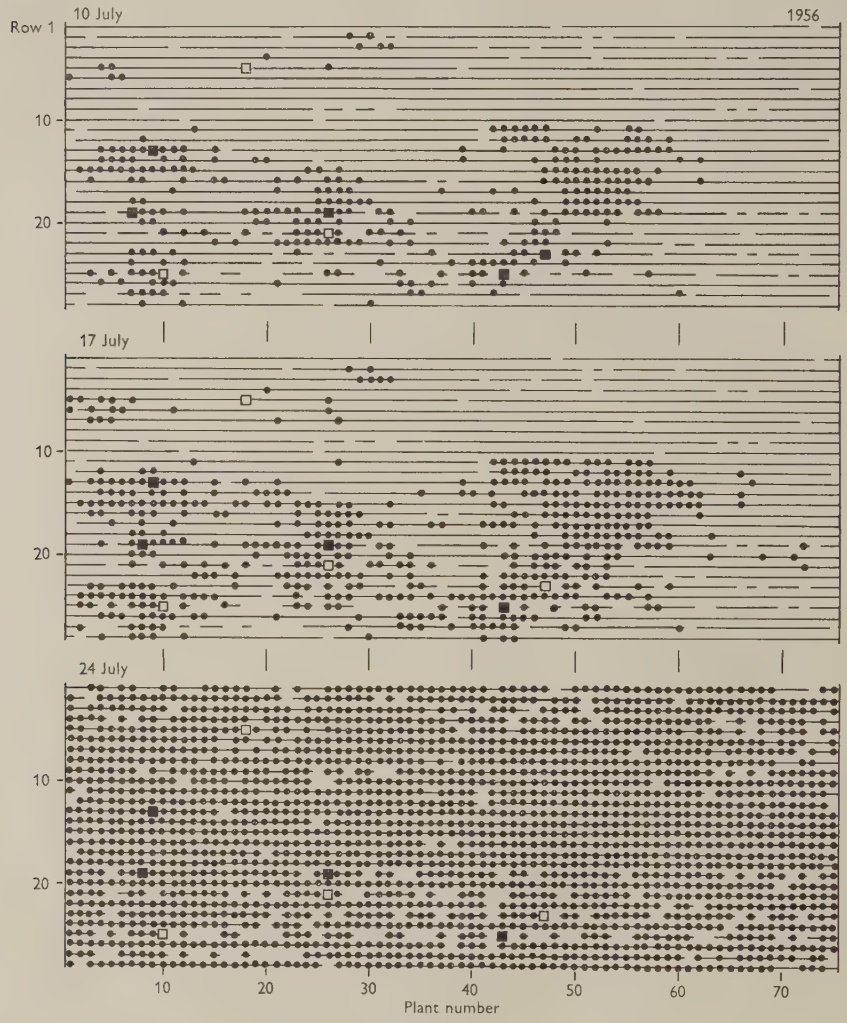


Fig. 3. (For legend see Fig. 2.)

Experiment in 1956

Few naturally infected tubers were available for an experiment in 1956, so in December 1955 over 900 tubers were inoculated and on 6 April 1956, 859 of them were planted among 1133 healthy guards (Table 4). Rows of diseased tubers, with

two healthy tubers at each end, alternated with rows of healthy tubers. Unless stated otherwise, this pattern was used in all subsequent experiments. Eight invaded stems were found in 1956, the first two on 12 June, and the last on 3 July (Figs. 2, 3). The first infected leaves were found on 19 June close to invaded stems and the attack developed much as in 1954 (Hirst, 1955*a*).

Experiments in 1957

The plot planted on 29 April 1957 was divided into three parts. In rows 1 to 10 (Figs. 4, 5), 276 Arran Pilot tubers infected on 25 February were planted in all odd-numbered rows except row 1; rows 11–19 were similar but the 276 blighted Arran Pilot tubers were in the even-numbered rows. Rows 1–10 were irrigated for reasons described later, rows 11–19 were not irrigated and can legitimately be regarded as a continuation of the series of phenological observations, together with the remaining rows 20–38 which contained 589 diseased tubers of which 556 were naturally infected.

Four invaded stems were found in rows 11–38, the first three on 16 July. Two were from artificially infected Arran Pilot tubers: the other two from King Edward tubers were the first in the series to come from naturally infected tubers. Although the first invaded stem was found more than a month later than in 1954 or 1956, the disease developed so rapidly that 84% of plants were infected by 7 August and on 16 August only two plants remained healthy (Figs. 4, 5).

Experiments in 1958

In 1958, two experiments containing blighted tubers, separated by a 17-row strip of healthy potatoes, were planted on 30 April. The southern, irrigated, plot is described below. The northern plot (Figs. 6, 7) was to continue phenological observations on the origin of blight attacks; it contained 700 King Edward and 140 Up-to-Date tubers, all infected naturally. To accommodate these diseased tubers within the area available, pairs of rows of diseased tubers were separated by single rows of healthy Majestic, otherwise the planting pattern was standard.

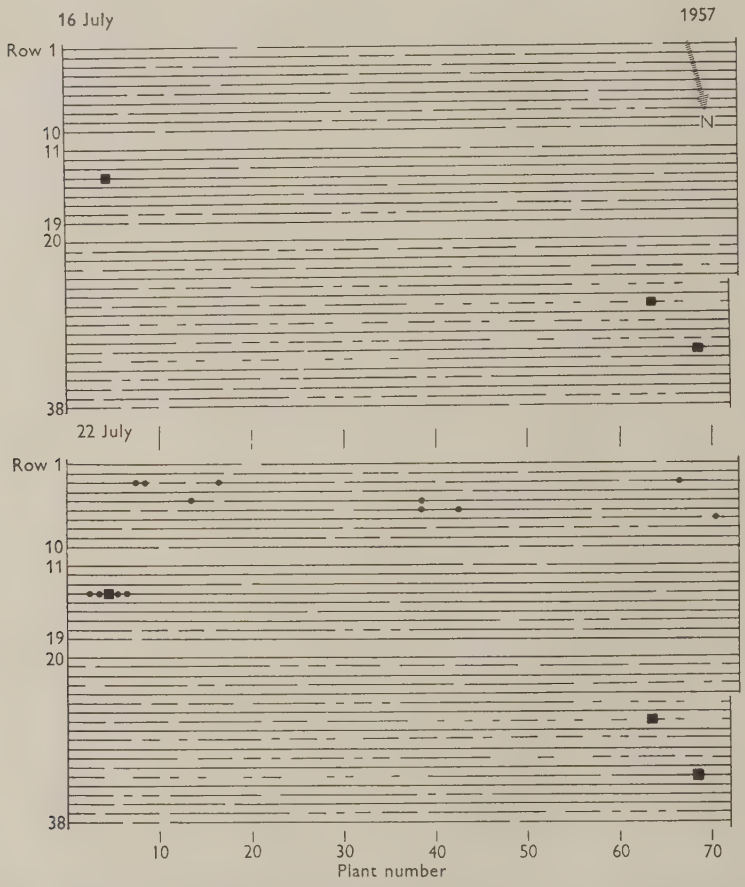
Invaded stems were found on seven plants all from King Edward tubers, the first on 19 June. The first isolated lesions on leaves were found on 3 July, mostly close to invaded stems but, unlike the previous years, there were a few exceptions. In the north-east corner of the plot a group of infected plants occurred without any associated invaded stem, the first time this happened in an unirrigated plot. The disease spread fast and 96% of the plants were infected by 29 July, all of them by 8 August.

Experiment in 1959

A plot containing 980 tubers was planted on 21 April 1959. The standard planting pattern accommodated 309 King Edward and twenty-one Majestic tubers all infected naturally. Its purpose was no longer primarily to estimate the frequency of invaded stems but to act as a reference plot indicating the earliest stages of the blight attack. Consequently, only diseased tubers thought likely to produce plants

were used, instead of any tubers with at least one live shoot, and because the results are not exactly comparable with earlier years they are not included in Table 4.

The results were interesting because the summer proved to be exceptionally hot and, in much of England, also dry. The statement that the rainfall at Harpenden between 1 April and 31 August totalled 11·06 in., or 97% of the average of this



Figs. 4, 5. Plans of plot in which blighted tubers were planted in 1957. Symbols as for 1956 (Figs. 2, 3), except that inactive or dead invaded stems are not separately distinguished. For description of subplots and planting pattern see text.

period, is somewhat misleading for much of it fell in heavy local thunderstorms in July, the effects of which were short-lived (see Fig. 10). Four stems from King Edward tubers having basal lesions thought due to *P. infestans* were found on 30 June (see Fig. 8, 23 July), three of them soon died. By 3 July the fungus had grown into the petiole of the basal leaf of the fourth (plant 31, row 11) and there

were isolated lesions on leaflets of other stems of the same hill on 16 July and on one nearby on 20 July (Fig. 8). Subsequent spread within the plot centred around this plant and 99% of the plants were infected by 21 August.

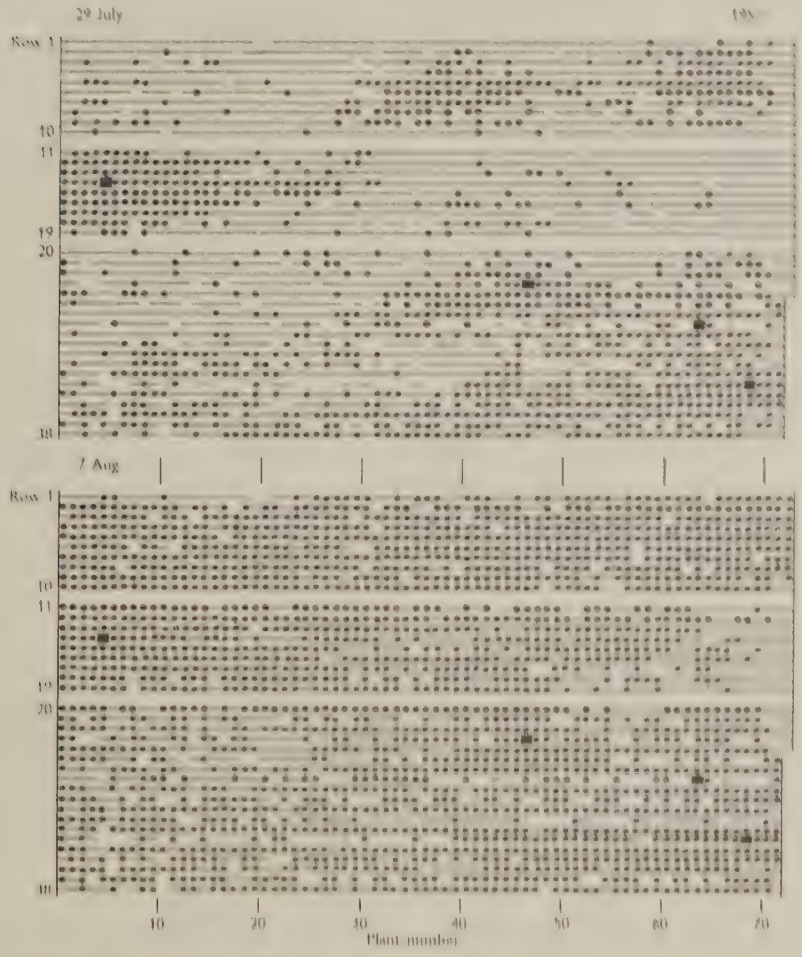


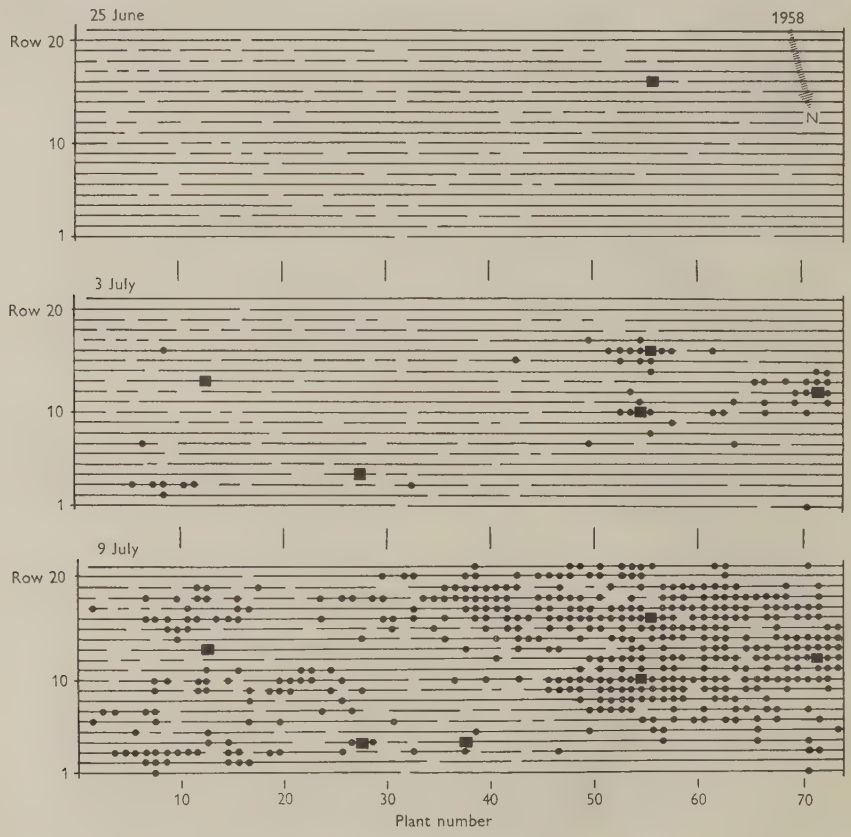
Fig. 5. (For legend see Fig. 4.)

An effect of soil moisture on the origin of blight epidemics

Earlier work and the experiments in 1955 (see Hirst, 1955*a*, and Tables 1 and 4) show that invaded stems are produced more often in glasshouse experiments than among farm crops. One reason is probably the shallower planting in pots, but there is evidence that high temperature and soil moisture favour their appearance. It

would be difficult to raise soil temperature sufficiently to test this among crops in this country but soil moisture can easily be increased by irrigation.

Rows 1–10 of the 1957 experiment, containing 276 artificially infected Arran Pilot tubers, were therefore irrigated with an oscillating spray line in the hope that they might produce more invaded stems than the equal number of similar tubers in the unirrigated rows 11–19. Sufficient water was applied, once or twice each week,



Figs. 6, 7. Plans of unirrigated plot in which blighted tubers were planted in 1958. Symbols as for 1956 (Figs. 2, 3), except that inactive or dead invaded stems are not separately distinguished. Rows 1, 2, 5, 8, 11, 14, 17, 20 and 21 and two guards at each end of all rows planted with healthy Majestic tubers, otherwise all tubers blighted.

to raise total precipitation to about 1 in. per week or twice average weekly rainfall (see Fig. 10). During the 9 weeks from 28 April to 29 June, 2·93 in. of rain fell and 5·86 in. were applied as irrigation. No irrigation was used in the next 3 weeks, to 20 July, because rainfall totalled 2·92 in. The experiment was inconclusive, only one invaded stem (plant 5, row 14, Fig. 4) was found, and this was on 16 July in the

unirrigated area. The experiment was not repeated, for it seemed more important to use the equipment in attempts to explain the discovery, on 22 July, of three groups of infected plants in the irrigated rows 1–10 where there was no recognizable source of inoculum. The discovery coincided with that of the first foliage infections around an invaded stem. Like these, the lesions were on leaves and varied in size, but three modal diameters suggested infections about 10, 6 and 4 days earlier.

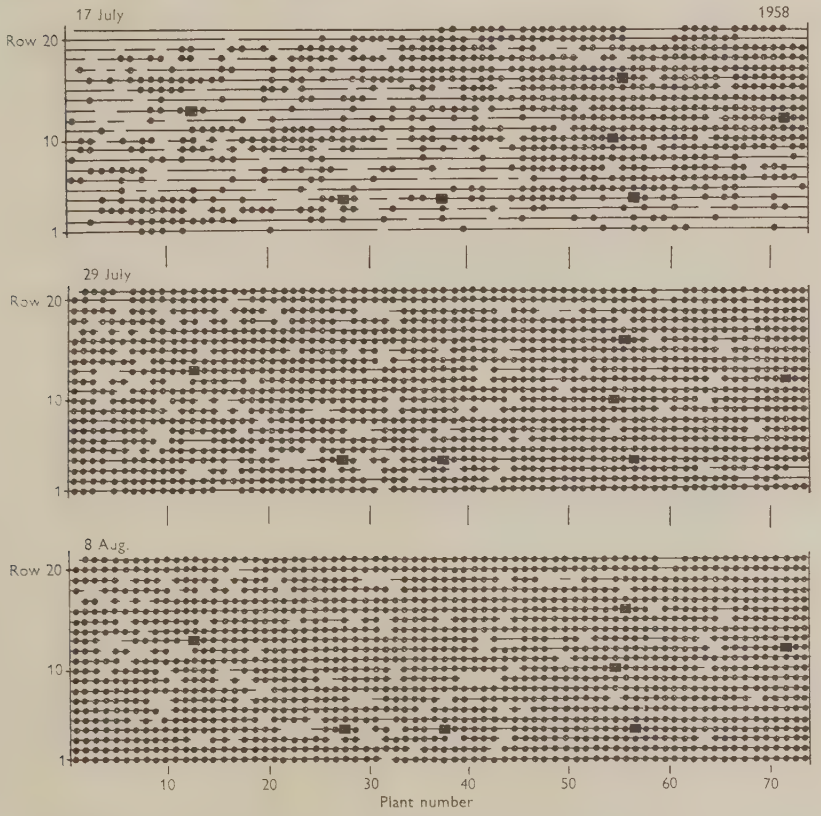


Fig. 7. (For legend see Fig. 6.)

Even the oldest of these lesions would have been minute on 16 July when the previous examination was made but their estimated ages suggest that, like those around the invaded stem, they were all the progeny of some earlier source rather than of one another. A careful re-examination of the plot failed to reveal any sources to explain these infections so it was necessary to assume that invaded stems had not been overlooked and try to find other possible sources of inoculum.

If irrigation significantly altered the ecoclimate, sporangia dispersed in air from

sources outside the plot might have infected plants in the irrigated rows, while failing to do so close to their source. No measurements were made because the plots were narrow but the density of foliage did not seem greatly increased by irrigation. In addition, any differences in soil moisture at the end of the treatment must have been masked by over 1·4 in. of rain which fell in the first 2 weeks of July.

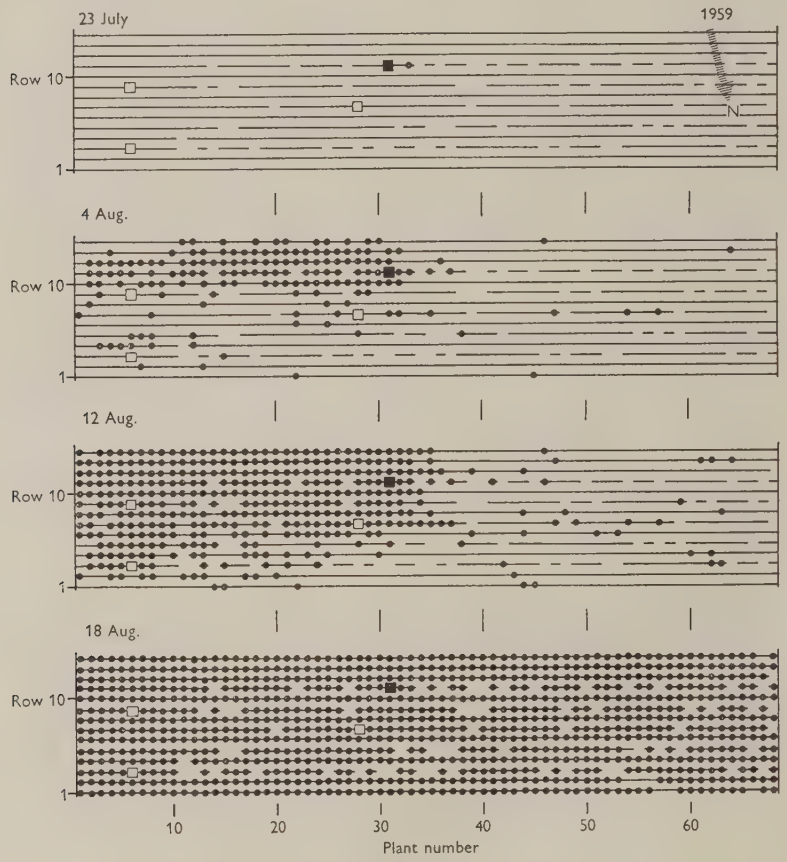


Fig. 8. Plans of unirrigated plot in which blighted tubers were planted in 1959. Symbols as for 1956; for full explanation see text.

Known sources of blight in the vicinity were limited to the invaded stems in other plots of the experiment, first found on 16 July, and spores were formed on them the following night, when the stem lesions extended approximately 3 in. above ground and so may have been active at least a week earlier. An alternative explanation is that sporangia or zoospores formed on buried blighted tubers may have reached the surface of irrigated soil, either through mycelial growth or transport in soil

water and then be splashed on to foliage which they infected. There is little evidence for any such activity in unsterilized soils from small-scale laboratory experiments, but it seemed worth while testing on a large scale, where the very large leaf area of a healthy potato crop might provide a sensitive indication of the activity of the fungus. For this test it was necessary to be able to distinguish any infections derived from underground sources of blight from those caused by air-dispersed spores. It was

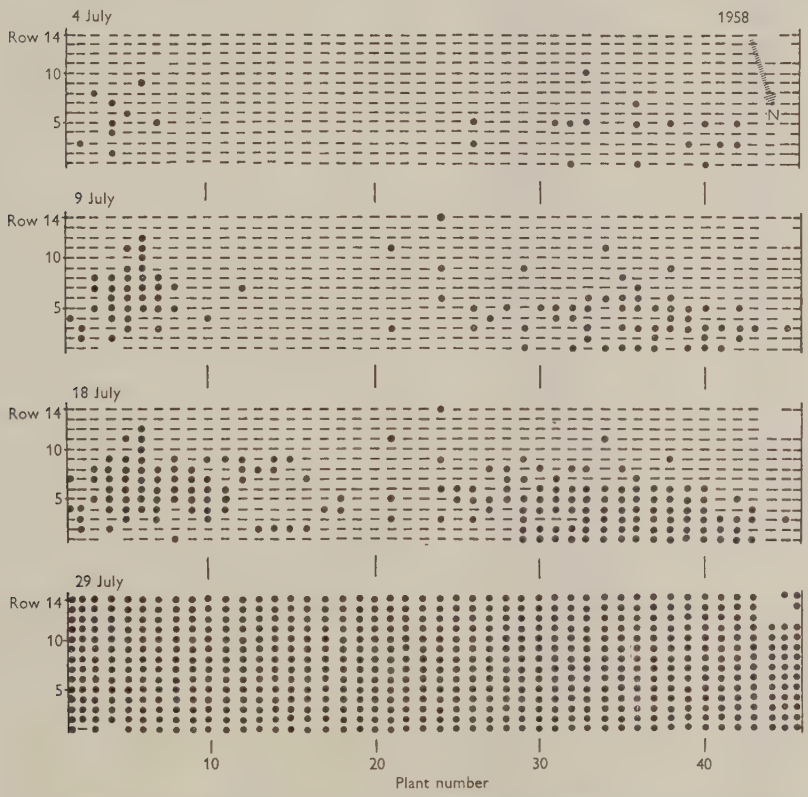


Fig. 9. Plans of irrigated plot planted in 1958 with healthy Majestic tubers and interplanted with diseased but eyeless Pentland Ace tubers except between two guard tubers at end of rows. Complete potential emergence represented by alternation of a symbol and single blank space. Further details in text.

decided to attempt this by using as the underground source an uncommon physiologic race recognizable by its pathogenicity. This biological tracer technique was used in 1958 when a 14-row plot was irrigated on the same regime as in the previous year. The healthy 'trap-plants' were all of the variety Majestic, they were planted 24 in. apart, except for two 16 in. apart, at each end of the rows. The source of

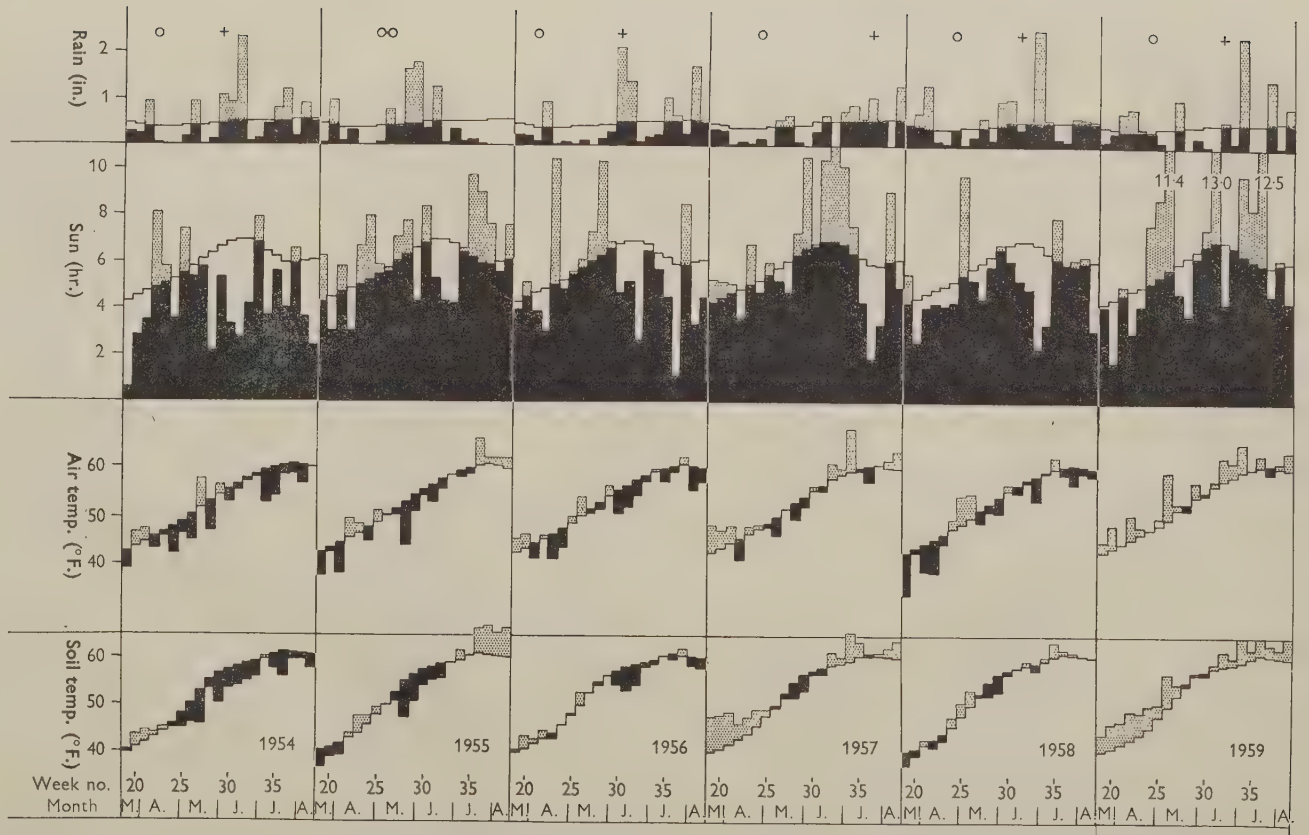


Fig. 10. Weekly means of total rain (in.), hours of bright sunshine per day, daily mean and soil temperature 1 ft. below ground at 09.00 G.M.T. at Rothamsted in the period from planting (○) through appearance of first invaded stem (+) to mid-August. Stepped curves common to all years show long-period weekly means, actual values of rain and sun are shown in black below the mean and dotted where they exceed it. Temperatures are shown as departures from the mean, excesses dotted and deficiencies solid black.

blight consisted of Pentland Ace tubers from which all the eyes had been punched by removing plugs, $\frac{1}{2}$ in. deep, with a cork borer. One of these tubers was planted between each pair of the wider-spaced healthy Majestic tubers. The Pentland Ace tubers in rows 1–9 were infected on 12 February with a culture isolated from blight lesions on Pentland Ace foliage in 1957 and hence capable of attacking hosts with R_3 and R_4 resistance genes. Rows 10–14 were planted likewise but with eyeless Pentland Ace tubers infected naturally in 1957; these may have contained Race 4 or Race 3, 4, both of which attack tubers of this variety. The 380 artificially and 200 naturally infected Pentland Ace tubers available were too few to interplant the west end of row 9 and the east end of row 14.

During the seven weeks, from 19 May to 30 June, 5·84 in. of rain fell, and 2·30 in. were applied as irrigation; in the last 2 weeks of the period there was nearly 3 in. of rain but no irrigation. No invaded stem was found in the plot but on 4 July, twenty-six of the Majestic trap-plants grown from healthy tubers were found to be infected (Fig. 9). These were only in the rows where artificially infected tubers had been planted or in row 10 which was adjacent to them. Most of the lesions were single and restricted to the lowest leaves, usually to the terminal leaflets lying close to or in contact with the soil in the base of the furrow. Infection of lower leaves of potatoes is common during the early stages of epidemics, but then lesions occur on any part of all lower leaves. In the plot containing invaded stems (Fig. 6) the lesions found on Majestic plants on 4 July were not limited to leaflets lying in the base of the furrow. It seems unlikely that this distribution was dictated by microclimate because higher leaves were infected on plants around invaded stems in the neighbouring unirrigated plots. Splashing of sporangia or zoospores from the soil surface or leaf contact with wet soil or water lying in the furrows at both ends of the irrigated plot on 27–28 June seems a more probable explanation for the limited distribution of lesions. To avoid disturbing the soil surface, this plot was not cultivated but weeded by hand. The area occupied by artificially infected Pentland Ace tubers (rows 1–9) had been weeded by late June but a dense growth of annual weeds in rows 10–14 had to be left until drier weather. Unfortunately there is no way of telling whether the lesions found on 4 July in rows 10–14 were very few because naturally infected tubers were planted there, or because the weeds prevented rain from splashing spores from the soil on to the lowest leaves, or because the weeds prevented the potato leaves touching wet soil in the furrow.

Half of a lesion from each of twenty-five plants was removed on 4 July and placed in damp Petri dishes overnight and most produced sporangia freely. Drops of sporangial suspension from each half-lesion were localized under four filter-paper discs on Pentland Ace leaves which are hypersensitive to the common Race 4 but develop lesions with Race 3, 4. The test was repeated on the remaining halves of the lesions 5 days later. Combined results from both tests showed that suspensions from twenty-one of the twenty-five lesions (84%) were pathogenic to Pentland Ace. Simultaneous tests from the original culture were 87% successful in attacking Pentland Ace leaves. By contrast no progressive lesions developed on Pentland Ace

from four replicate suspensions of four isolates from King Edward and one from Majestic plants infected close to invaded stems in the nearby experiment, although when similarly tested on the varieties from which they came, 80% caused progressive lesions. The high proportion of isolates pathogenic to Pentland Ace among those tested from the irrigated plot, where Race 3, 4 had been introduced, and their absence from the adjoining plot seems strong evidence that infections which developed in the irrigated rows were derived from fungus introduced below ground on tubers incapable of producing stems.

A similar experiment in 1959 gave inconclusive results, first because some tubers were disturbed during early cultivations and, secondly, because it proved impossible to recognize the cultures used to infect the eyeless tubers among the isolates later found infecting basal leaves. Subsequent tests indicated that neither the original cultures nor isolates recovered from the trap-plants had pathogenicities proper to the physiologic races which were supposed to have been used. Despite this, their pathogenicity differed from the common Race 4, either from cultures or spreading around invaded stems in a plot where naturally infected tubers had been planted. Therefore, although migration from subterranean sources cannot be proved the possibility that it happened need not be rejected. Isolated lesions on leaflets close to soil in the furrows were again found, so a brief account of the experiment seems justified. The planting pattern and irrigation regime were as in 1958, 660 eyeless tubers infected in early April were planted (21 April). Between planting and 16 July, when the first infected leaves were found 5·58 in. of rain fell and 5·83 in. were applied as irrigation. On 20 July, thirty-four of the intervening Majestic trap-plants bore lesions on basal leaves but no similarly situated lesions were found in an area irrigated but without diseased tubers.

The frequency of invaded stems

The experiments where live but blighted tubers were planted as normal seed to measure how often invaded stems were produced amply confirmed their importance but represented only one of the possible ways of starting epidemics. To assess their real contribution it would be necessary to know how often crops are infected by fungus over-wintering in ground-keepers and in clamps or stores whence it too often finds its way to cull piles or other rubbish heaps in spring.

The occurrence of ground-keepers was studied by Doncaster & Gregory (1948). In the first two arable crops immediately following potatoes they usually found thousands of volunteer plants per acre and some persisted in the fourth and fifth crops. Their data contain no information on the proportion of ground-keepers which were blighted. If diseased tubers are rejected during picking the proportion is likely to be higher than in the harvested produce. The severity of frosts and the rate at which tubers are killed by microbial rots determine how many blighted tubers survive the winter. In November 1956 we planted 100 blighted Ulster Supreme tubers, half at 4 in. and the remainder 8 in. below the surface. By June 1957 fourteen plants had emerged and none had invaded stems. On 28 November 1957,

311 naturally infected tubers of eight varieties were planted at 4 in. and 343 at 8 in. Most of the tubers were of the three varieties King Edward (190), Majestic (139) and Up-to-Date (235), almost equal numbers of which were planted at each depth. In the summer of 1958 plants developed from 31·5% of the shallow- and 21·6% of the deep-planted tubers. One naturally infected King Edward tuber (0·15% of the total) planted 4 in. deep produced six stems, two of which were found on 4 July to be invaded by *P. infestans*. Later the fungus spread from these to infect foliage. In December 1958 naturally blighted tubers were again planted at the same depths but fewer shallow-planted tubers produced plants, presumably because frost penetrated deeper than in the previous mild winter. Of 104 Up-to-Date tubers planted at 4 in., 1·9% produced plants and of 148 planted at 8 in. 10·8% produced plants. Similarly, of ninety-six and eighty-six King Edward tubers planted at the two depths, none at 4 in. produced plants, whereas 18·6% at 8 in. did. When the plants were removed on 9 June none was visibly infected. The tests show that blighted ground-keepers can produce invaded stems and start epidemics but suggest that they do so rarely. This agrees with observations that volunteer potato plants are not found blighted before neighbouring crops. Even the rare occasions when invaded stems appear on volunteer plants may be of little importance, for in areas, like England, where crop rotation is usual there would seldom be plentiful susceptible foliage close by, on which the fungus might multiply.

Blighted tubers from stores or clamps probably stand more chance of starting epidemics. They are protected from frost until discarded on heaps where those still alive often produce dense foliage which encourages the formation of blight-favouring ecoclimates and rapid multiplication of the fungus. Thus a source of sporangia may become sufficiently powerful to endanger crops at a distance early in the season. Foci of this type occur regularly (Bonde & Schultz, 1943, 1944; Large, 1956), but it is as difficult to measure their frequency and effect accurately as it is to understand why so many are allowed to develop.

Similarly, there is little information of the actual proportion of blighted tubers in seed stocks. At harvest most crops have few diseased tubers but a small proportion may be heavily infected (Large, 1958). Even examining enough harvested crops of seed tubers to provide a representative sample may be of little value if, during the preparation of seed consignments, proper care is taken to reject diseased tubers. To accumulate the necessary information there is no alternative to a careful search through large samples of many seed stocks just before planting, and this has rarely been attempted (Wallin, 1956).

From the results of our experiments and subsidiary observations it is, however, possible to make some estimate of the number of blighted tubers required to produce a single invaded stem. After collection naturally infected tubers were washed, dried and stored on trays in a frost-proof but unheated building. Shortly before planting the tubers were examined and only those having at least one live shoot were retained for planting. Records of the proportion rejected varied from 14 to 72% but the mean for nearly 2000 tubers collected was 47% (Table 2). In practice the proportion of blighted tubers alive at planting must depend considerably on the

date when the seed sample is prepared and on the storage conditions. Death of the fungus within tubers may in part account for the small proportion of them which produce invaded stems. Tubers of six susceptible varieties, which were not surface sterilized, were infected on 6 December 1955 with aerial mycelium from pure cultures. At intervals over the next 6 months samples were cut and slices incubated in damp Petri dishes, and production of sporangiophores was used to indicate the presence of live *P. infestans*. Table 3 shows that, except in Home Guard, the proportion that spored declined little in the first 3 months but fell to less than half at 6 months. Although temperatures during the last 3 months of this test, ending in June, may have been higher than during the normal 6-month interval between infection and planting, it seems probable that many blighted tubers may no longer be dangerous at planting time because *P. infestans* often fails, as in this test, to compete with other rotting organisms.

Table 2. *Proportion of naturally infected tubers rejected before planting*

	Year		
Variety	1953–4	1954–5	1956–7
Majestic	20/82, 24%	54/114, 47%	—
King Edward	24/53, 45%	—	325/802, 41%
Ulster Cromlech	—	29/115, 25%	—
Ulster Chieftain	98/197, 50%	10/71, 14%	—
Arran Pilot	—	—	147/208, 71%
British Queen	—	—	53/77, 69%
Up-to-Date	—	—	125/174, 72%
Total	—	—	885/1893 = 47%

Table 3. *Percentage of tubers containing viable (sporing) blight at monthly intervals from inoculation on 6 December* 1955

Variety	No. of tubers sampled each month	Jan.	Feb.	Mar.	Apr.	June
Ulster Cromlech	20	100	100	95	90	10
King Edward	20	100	100	100	90	40
Majestic	20	95	100	80	65	15
Chancellor	20	60	90	90	70	50
Dr McIntosh	10	100	90	100	70	0
Home Guard	6	100	100	50	0	—

Diseased tubers may fail to produce plants or invaded stems because the eyes are killed, the sprouts too weak to emerge, or because the fungus dies or fails to advance up the growing stem. These factors have not been analysed individually because their effect on the production of invaded stems is incorporated in the percentages quoted in Table 4 which are based on total tubers planted rather than on plants produced. Between 1954 and 1958, 3200 blighted tubers were planted in unirrigated plots. These produced twenty-one invaded stems (0·64%), slightly more from those artificially infected (0·79%) than from those diseased naturally (0·52%). The data do not justify conclusions about years or varieties because of the hetero-

geneity of tubers planted in the various years. Thus, there is no reason to suppose, as Table 4 suggests, that naturally infected King Edward tubers really produce more invaded stems than those infected artificially. It is clear, however, that experiments of this type can be relied upon only when they contain several hundred tubers. Except in 1955, most invaded stems caused local outbreaks of blight, but to produce one invaded stem it was necessary to plant 150 live but blighted tubers.

TABLE 4. *Fraction of diseased tubers which produced invaded stems.* (*Figures in brackets for artificially infected tubers, others for naturally infected tubers, overall percentages in heavy type*)

	Year					All years percentage with invaded stems
Variety	1954	1955	1956	1957*	1958*	
Majestic	0/47	0/60	—	—	—	0 } **0·2**
	—	[0/60]	[1/281]	—	—	[0·3] }
King Edward	0/13	—	—	2/273	7/700	0·8 } **0·7**
	—	[0/60]	[1/284]	—	—	[0·3] }
Ulster Cromlech	—	0/90	—	—	—	0 } **1·4**
	[2/117]	[0/60]	[6/294]	—	—	[1·7] }
Ulster Chieftain	0/69	0/60	—	—	—	0 } **0**
	—	[0/60]	—	—	—	[0] }
Arran Pilot	—	—	—	0/39	—	0 } **0·6**
	—	—	—	[2/309]	—	[0·7] }
British Queen	—	—	—	0/24	—	**0**
	—	—	—	—	—	
Up-to-Date	—	—	—	0/120	0/140	**0**
	—	—	—	—	—	
	Percentage with invaded stems					
Naturally infected	0	0	—	0·4	0·8	**0·52**
Artificially infected	[1·7]	[0]	[0·9]	[0·7]	—	**[0·79]**
Total	**0·8**	**0**	**0·9**	**0·5**	**0·8**	**0·64**
	Total tubers in plot (including healthy)					
—	738	4004	2092	2030	1554	

* Unirrigated plots only.

Even if this number is doubled to allow for death of blighted tubers before planting it seems that as few as 0·01 % of blighted tubers in the seed stocks would be sufficient to produce the frequency of initial sources claimed by van der Zaag (1950), who estimated that there was one 'primary focus' in every square kilometre (247 acres) of potatoes, an area which would require roughly 3 million seed tubers. To prevent such a small contamination would be more than could be expected of any commercial inspection and beyond the levels of control usually achieved in plant pathology.

Migration of the fungus from over-wintering substrate to first foliage infection

Unfortunately we could not remove the plants with stem lesions to see whether there was mycelial continuity between the lesion on the stem and its parent tuber, for this would have spoiled our phenological observations. Earlier workers have suggested that there may be no such continuity, and that shoots with lesions above ground become invaded below ground indirectly through spores produced on the tuber or invaded sprouts which die before they emerge (de Bary, 1876; Jensen, 1887; Josifović & Sutić, 1952; Naoumova, 1939). Epidemiologically it is more important to know how often invasion succeeds in producing a lesion above ground than precise details of the route it takes. But, if invasion is indirect it is important to know whether this is the only activity of the fungus in the soil.

The mode of origin of the epidemics in irrigated plots containing many tuber cultures of *P. infestans* suggests the fungus may be able to move in soil. The eyeless tubers in these plots served merely as a convenient selective and persistent medium for culturing *P. infestans* underground, and any inert medium equally capable of supporting the fungus against competitors in the soil could presumably have been substituted. Our methods to date are open to criticism for at least five reasons. First, the lack of sufficient irrigated controls. Secondly, they do not prove the size of food base which is required or whether naturally diseased tubers which fail to produce plants are dangerous, although the infections on 3 July 1958 around plant 9, row 3 (Fig. 6), may have originated from such tubers for the first lesions found were on leaves in the base of the furrow. Thirdly, proof of origin requires a reliable tracer technique, free from the uncertainties experienced in 1959. Fourthly, it would have been better to have irrigated to maintain a particular water deficit than to apply a fixed amount, irrespective of variable losses by evaporation. Fifthly, the use of healthy trap-plants, although essential to demonstrate that the fungus was present, did not allow individual sources of infection to be identified as was possible when the initial source was an invaded stem.

The first lesions in the irrigated plots were often discovered after the first invaded stems but usually coincided with the earliest spread around them. Foci developed in irrigated plots, therefore, at the same time as around invaded stems in unirrigated crops. This obviates the need to have different theories underlying potato blight forecasts depending on the origin of the initial inoculum. Equally it excludes any possibility of identifying the origin of first infections by phenological differences. Until the origin of attacks after irrigation is established, we can only guess how the fungus migrated from buried tubers to infect foliage. Movement by soil animals, cultivations or mycelial growth must all be regarded as possibilities, but we think the most likely explanation is that zoospores or sporangia were moved by soil water.

Irrigation which began soon after planting was discontinued in our experiments, because of wet weather before the lower leaves became infected. The interval between ending irrigation and the discovery of lesions was 4 weeks in 1957, 2 in 1958 and 1 week in 1959. Particularly in the last two years, the size of the first

lesions when found suggested that infection accompanied heavy rainstorms when soil in the furrows became waterlogged. In 1957 such happenings were not noted in detail, but the records show two periods when more than ½ in. of rain fell in 24 hr. (Table 5). The hypothesis we intend testing in the future is that in irrigated soil the fungus grows on or from the buried tubers and forms spores in soil, but that these reach healthy foliage only when they are flushed out of the ridges by heavy rain.

TABLE 5. *Relation of irrigation and storms to appearance of first lesions in irrigated plots*

Year	Last irrigation in week ending	Heavy rain following end of irrigation	First lesions found
1957	28 June	29–30 June (0·6 in.), 11–12 July (0·6 in.)	22 July
1958	30 June	26–27 June (1·48 in.)	4 July
1959	21 July	10–11 July (2·34 in.)	16 July

THE PHENOLOGY OF INVADED STEMS

In all years except 1955 invaded stems were found several weeks before disease 'outbreak' (Large, 1953). The date when the first was seen ranged from 28 May in 1954 to 10 July in 1957 and was 45 days after planting in 1954 (when tubers were not planted in ridges), 67 in 1956, 78 in 1957, 50 in 1958 and 71 days in 1959. Thus the interval between planting and the discovery of invaded stems averaged 62 days for the first or 73 days for all of them. The time between planting and the date when sprouts of half the tubers had emerged varied from 3 to 6 weeks, but there was usually a further delay before invaded stems were found, presumably the time occupied by the growth of the fungus approximately 20 cm. up the stem between the parent tuber and the crest of the ridge. The fungus was found to grow about 1 cm. per day in the stems of glasshouse-grown potato plants (Keay, 1953; Hirst, 1955*b*), but is unlikely to be so active in potato ridges in spring.

The occurrence of the first invaded stems marks the real start of blight attacks and it would be helpful if it could be predicted from weather records. In Fig. 10 the stepped lines represent the long period weekly means for total rain, hours of bright sunshine per day, daily mean air temperature and soil temperature at 09.00 hr. 1 foot below ground. For sun and rain the actual values are shown, in black below the mean and dotted where they exceed it. The temperatures are shown as departures from the mean, excesses are dotted and deficiencies solid black. Circles and plus signs mark, respectively, the week of planting and discovery of the first invaded stem. In years when the first symptom was found in May or by mid-June there was or had recently been a cool dull week with an inch of rain (weeks 29, 30 and 31 in 1954, 1956 and 1958 respectively). In 1957 the first invaded stems were not found until mid-July during week 36: the first week during the season when the above condition applied. On this basis invaded stems should have been found in May or by mid-June in 1955 or perhaps in late-May in 1959. In fact none was found in 1955 and the first not until the end of June (week 25) in 1959, when there was slightly over ½ in. of rain, fewer than 4½ hr. sun per day, but above

average temperatures. In both years it seems possible that weather favouring the extension of the fungus above ground may have occurred early in the season before sufficient time had elapsed since planting for it to have reached the soil surface. Diseased tubers were planted late in 1955, the quickest recorded appearance of an invaded stem would not have been expected until 17 June and the first on average not until 29 June, by which time the weather had become warm and dry. Similar dates in 1959 would be 10 and 22 June, so the potentially favourable week 20 in the second half of May would have been too early but the invaded stems did appear at the end of week 25. Apart from reviving the 'dormant mycelium theory' (Massee, 1906; Keay, 1953, 1954) there is no reasonable explanation why the appearance of invaded stems should be associated with cool, dull and wet weather. Certainly the growth of the fungus within stems is not limited to wet weather (Clayson & Robertson, 1956) nor is it usually devoid of visible necrosis.

The development of foci and their effect on surrounding crops

Grainger (1956) has suggested that potato plants resist infection by *P. infestans* in the middle of their growing season, but our experiments show that, once the first lesions occurred on foliage, newly infected plants were found at almost every weekly inspection. Admittedly, the new infections were few, but because they were generally very close to the known sources of the fungus it seems probable that they were limited by scarcity of spores rather than by host susceptibility and would have been more numerous and widespread had the source of spores been larger. The rate of fungus multiplication varied from year to year, no doubt because of differences in the frequency, duration and favourability of infection weather, as well as in the strength of the fungus source and the pattern of its dispersal.

As the exact meteorological conditions required for infection among crops have not been defined, we have used a simple criterion of persistent dampness merely to indicate changes in the frequency of weather which possibly permitted infection. In Fig. 11 black dots mark days when relative humidity was not less than 90% for longer than 15 hr. as shown by instruments between potato ridges but level with their crests (Hirst & Stedman, 1960). Vertical strokes beneath the lower line of the pair for each year in Fig. 11 indicate days when new lesions were observed in the plot. Precise agreement between the two patterns of occurrence is not to be expected because the dots do not accurately define infection weather and at first the difficulty of finding a very few new lesions was such that an effective search could not be made every day. This became possible once foci of infected plants showed where lesions were most likely to appear. The dots then refer to flushes of lesions, with necrotic centres about 2 mm. in diameter, which depending on the temperature indicate infection 3–6 days previously. Nevertheless, the diagram supports our contention (Hirst & Stedman, 1960) that infections, at first only sporadic, become frequent and massive when the ecoclimate is modified by wet weather accompanying dense foliage, for there is general agreement between the density of dots and strokes. Dry weather and lack of the fungus prevented new lesions

appearing until mid-August in 1955 and mid-July in 1957. Sporadic infections occurred early in 1954 and 1956, and the disease developed much more slowly than in 1957 and 1958 when continued high humidity in the crops immediately followed the appearance of the first invaded stems.

The typical development of potato blight, through foci of plants more heavily infected than their neighbours to a general epidemic, implies methods of local and distant dispersal. Both dispersal patterns were evident in our experiments and although unproven, it seems possible that most local dispersal is by water-borne spores, whereas air-borne spores are more important in distant dispersal. Records made during the development of foci in 1956 can be used to illustrate this. When more than one infection period occurs within a week, the extent of spread during each can be determined only when the weekly examinations record the distribution

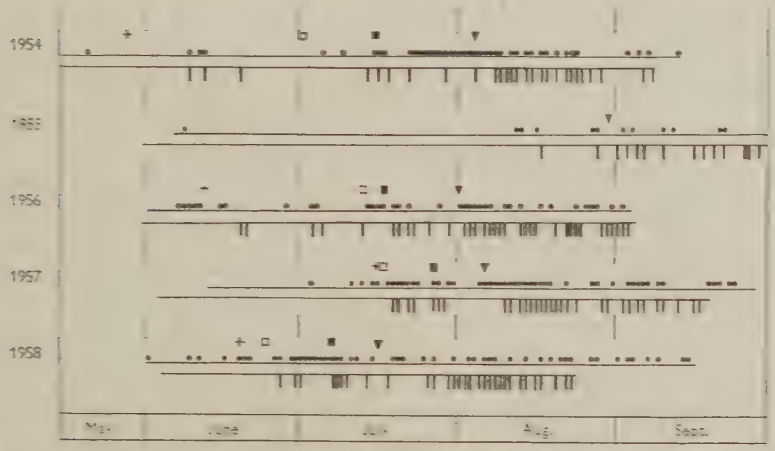


Fig. 11. Dates of discovery of first invaded stem (−), of first (□) and second (■) forecasts (see text) and of regional blight outbreak (▼). Black dots indicate days when relative humidity among the plants was not less than 90 %, for at least 15 hr., the line beneath indicates period instrument was operating. Lower line indicates period of search for new lesions and vertical strokes the dates on which these were found.

of lesions of different age by dividing them into different size groups. This procedure was possible in the first four examinations in 1956 (19 and 26 June, 3 and 10 July) because occasions of spread were sufficiently separated to be readily distinguished by lesion size (see Hirst, 1958). Flushes of minute lesions appeared on 19–20 June, 3–4 and 7 July, and are plotted in Fig. 12 A, B and C. Some discrepancies occur between these diagrams and the weekly examinations plotted in Figs. 2 and 3, because the dates of inspection happened to coincide with the appearance of minute lesions, so some plants with only one or two infections were not found until the next inspection, when the lesions had grown and become conspicuous. The fourth and fifth flushes of lesions appeared on 13 and 19 July and

were visible when the crop was inspected on 17 and 24 July. As some plants were already quite heavily infected by this time the complete distribution of those flushes could not be plotted, so Fig. 12D shows only plants which were first infected on either of these occasions.

During the first infection period the only source of active fungus in the area represented on Fig. 12 was the invaded stem of plant 47, row 23. The fungus spread mainly south-westwards and infected plants at least twelve rows distant, an

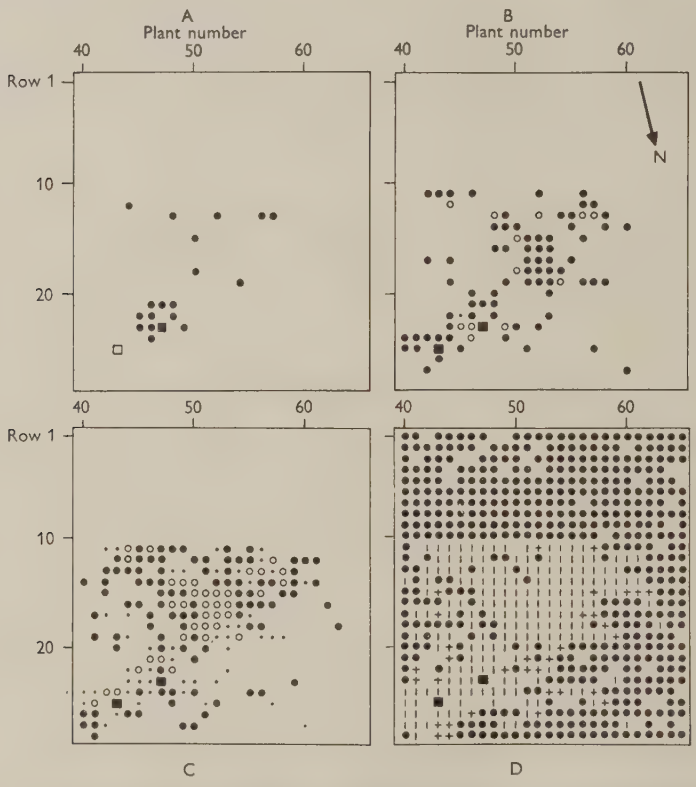


Fig. 12. Plans of part of the plot planted with blighted tubers in 1956 showing the distribution of all plants infected in each of the first three infection periods, A, B, C, and only of plants initially infected in the fourth and fifth infection periods, D. Symbols in A, B, C represent active (■) or inactive (□) invaded stems, plants previously infected but not re-infected (•), plants previously infected and re-infected (○) and plants newly infected (●). In D, plants previously infected (|), plants initially infected on fourth occasion of spread (+) and on fifth (●).

unusually long way from so small a source (Fig. 12A). The second flush of lesions (Fig. 12B) mainly appeared close to plants already infected or to the stem of plant 43, row 25, which had by then developed a lesion, and the proportion of

infections remote from known sources was small. Before lesions of the second flush were big enough to produce spores, a third infection period occurred; as would be expected the third flush of lesions were therefore again close to plants infected on the first occasion and not around isolated infections of the second flush (Fig. 12C). The fourth flush of lesions was probably small and certainly only extended the focus slightly (Fig. 12D), unlike the fifth which spread the disease to the extremities of the plot and probably beyond them. In this example, although only eighteen plants were infected in the first period, they established the limits of a focus which was not greatly expanded by the next three waves of infection. The local spread during the second, third and fourth infection periods involved many more plants, and an increase in lesion number which was even more than proportionally greater. The source of sporangia was then enough to infect almost every plant in the plot during the fifth infection period when distant spread once again occurred.

Summarizing our observations on the origin of attacks, lesions occur during early infection periods only on plants close to the initial source of fungus. Subsequent spread intensifies this focus and expands it until it contains one hundred or two hundred infected plants. Then, given conditions suitable for distant dispersal, spread occurs beyond the focus and to adjacent crops. The time for spread from an initial lesion to have effect at a distance depends both on the frequency of infection periods and on their duration and the dispersal patterns they permit.

Blight appeared earlier in plots within 100–200 yd. of the foci than in the other crops within 1 mile radius, for no difference was detectable between these and crops even farther afield. Since 1950, the 30 acres of potato crops each year on the 500-acre Experimental Farm have been examined for early foci of potato blight; one was found in 1951, and one in 1959. Except for these, crops have generally become infected uniformly, suggesting that the fungus originated at a distance so that the spore cloud had become uniform in concentration. We have no evidence that foci in a $\frac{1}{4}$-acre plot materially affect the date of blight outbreak in crops more than half a mile distant. Neither can we say whether the apparently uniform introduction of spores into most crops results chiefly from many widely scattered foci, within perhaps a 10-mile radius, or crops already heavily infected in other parts of the country.

The origin of blight attacks in relation to forecasting and control

Methods of forecasting potato blight outbreaks meteorologically have either been developed empirically (Beaumont, 1947; Cook, 1947) or from theoretical models of the weather required for infection (Bourke, 1953, 1955). Although the models are based on laboratory experiments, none has been shown to define the requirement accurately by infection experiments among crops, but experience suggests that several provide workable approximations. One of the difficulties of methods depending on temperature and humidity measurement has been the occurrence of weather fulfilling the criteria early in the growth of potato crops, without any associated appearance of the disease. In practice such premature warnings have

either been ignored as in the 'Zero-date' concept (Beaumont, 1947; Large, 1956), or accumulated up to a predetermined number when subsequent alarms are regarded as important (Bourke, 1955). The authors of the forecasting methods have either surmised or been reticent about the activity of the fungus at the time warnings are given. In fact, their methods must assume either that the fungus first appears above ground during the weather which leads to the warning or is there already and that the weather causes it to become ubiquitous.

In our experiments planting many blighted tubers undoubtedly increased the frequency of initial sources but there is no reason to suppose that it altered the phenology of the disease in unirrigated plots. Provided that there were sufficient live blighted tubers, the appearance of the fungus preceded blight outbreak by 1–2 months in 5 years out of 6. Development of the foci, summarized in Fig. 13, shows that there was only a little spread at first, close to invaded stems. At the end of this phase, the curve of percentage plants infected in our plots took a sharp upward turn as the spread became both frequent and massive. This point of inflexion marks a particularly critical phase in the development of the subsequent epidemic. Meteorological records show that it accompanied the development of a blight-favouring ecoclimate. It was soon followed by the first detectable spread from the experiments to adjoining plots. This infection of surrounding crops often occurred during the Beaumont periods which elicited a blight forecast. If similar dispersal occurs round all natural foci at this stage, the fungus will already have been introduced into many crops by the time the warning is promulgated, although it may require further multiplication to reach the stage defined as an outbreak. In plots with foci, half the foliage was destroyed 16, 20, 25, 12 and 20 days earlier than in unsprayed crops of Majestic elsewhere on the farm. These differences are comparable to the prolongation achieved by two or three sprays with protective fungicides and must represent how much the potential development of blight was retarded while crops without initial sources became infected. At present fungicidal control of potato blight is entirely protective, so to achieve the maximum effect the first application must be made before the fungus is introduced into the majority of crops.

It is interesting to compare the development of foci with the timing of forecasts issued for England and Wales by the Ministry of Agriculture. Forecasts were based on the occurrence of Beaumont periods (almost entirely measured in louvred screens 4 ft. above ground), knowledge of local climates, the growth stage of crops, early records of diseased plants, the weather outlook and the prospect of a reward from spraying (Large, 1953, 1956). In dry years, like 1955, warnings may not be issued, for avoiding unnecessary spraying was regarded as equally important as good timing when they were required. In most years two predictions were issued to the press and broadcasting services on the dates shown in Figs. 11 and 13. The first (hollow squares) generally predicted outbreaks in other areas but occasionally suggested the need to spray the more forward crops of susceptible maincrop varieties in Hertfordshire. Except in 1959, the regional warnings always preceded blight outbreak at Harpenden. The warning of outbreak in other areas preceded

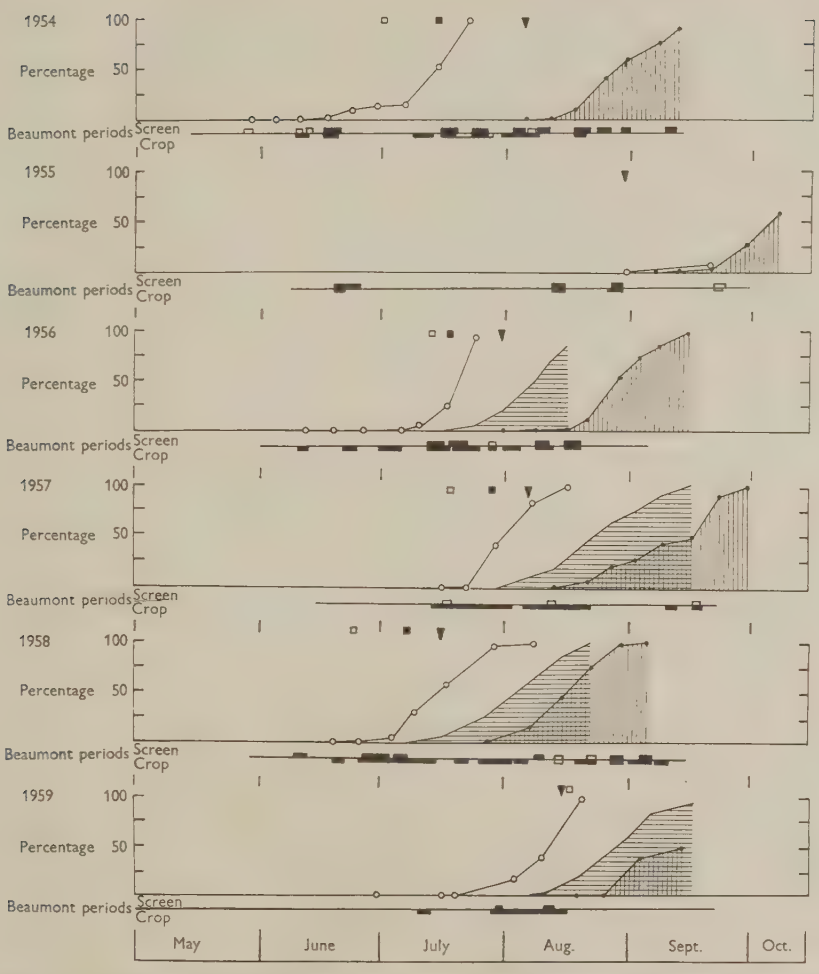


Fig. 13. Percentage plants infected (open circles) and destruction of foliage (horizontal shading) in blighted tuber plot related to regional epidemic (vertical shading or solid circles) and forecasting. Estimates of percentage destruction of foliage by blight according to British Mycological Society Key (Anon., 1947). Periods satisfying Beaumont's forecast-criteria as solid black rectangles, near-critical periods as hollow rectangles, recorded from instruments in screens or in crops respectively above or below a line which indicates period when instruments were operating. Arrow heads show date of regional blight 'outbreak' and hollow or solid squares the first and second forecasts of outbreak (see text).

the upward inflexion in the curve of percentage plants infected in our experiments. By contrast the operative warning for the area followed this critical stage of the attack and so probably came too late for control of the first introduction of the fungus into crops lacking initial sources. Failure to indicate this introduction, the critical point of epidemic development, becomes almost implicit in forecasting systems where notice of favourable temperature and humidity conditions is restricted to a period of less than a month before the normal season of blight outbreak. Under these circumstances the most valuable function of forecasts is probably to prevent wasteful fungicide applications.

To issue warnings of the outbreak of potato blight in epidemic proportions on a national scale is a complex problem and cannot be expected to cater for specialized localities or crops. In England and Wales their timing has been based on the belief that if there are to be only two or three sprays, the best time to apply the first is at or just before blight outbreak (Large, 1952). Certainly, early sprays may often be wasted because their residues, particularly of the newer fungicides, may be removed and new foliage produced before the disease appears. Economic considerations and recollection of severe losses in the past will inevitably decide the compromises of spray timing between what is epidemiologically desirable and expedient in practice. Nevertheless, forecasting methods should supply information equally suitable for early or late timing of the first sprays. There is also a need for experimental study of the cost and benefit from applying the first spray at different phases in the neglected first half of blight attacks as well as in the more spectacular epidemic phase which follows blight outbreak.

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STUDIES ON *GLOEOSPORIUM MUSARUM* CKE. AND MASSEE CAUSING STORAGE ROTS OF JAMAICAN BANANAS

II. SOME FACTORS INFLUENCING ANTHRACNOSE DEVELOPMENT

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Experimental inoculations indicate that several factors may influence the extent to which anthracnose, caused by *Gloeosporium musarum*, develops on bananas following wound-infection. Severity of disease increases with increasing 'grade' of maturity, 'full' fruit being more seriously affected than 'thin' fruit. The evidence suggests that Gros Michel fruit is less susceptible to this form of rot than fruit of the Lacatan or Robusta varieties. The amount of *Gloeosporium*-rot on fruit imported into the United Kingdom undergoes marked seasonal variation, being greatest during autumn and winter.

The presence of perforated 'Polythene' bags during cold storage (55° F.) appears to favour slightly the development of disease. Bagging had no significant effect on rotting during the subsequent ripening period. After wound-inoculation, disease incidence and severity decreased appreciably with increasing periods of storage at tropical temperatures (76–87° F.). Such a decrease was evident even when the humidity at the surface of the fruit was maintained at a high level (92–100 %).

In several shipping trials, a post-harvest fruit-dip treatment with nystatin (200–400 p.p.m.) effected 40–70 % control of anthracnose.

Introduction

Infection by *Gloeosporium musarum* Cke. & Massee through small skin injuries present on harvested immature banana fruits results in the development of characteristic lesions during the subsequent transport and ripening periods (Meredith, 1960). Lesions are typically lenticular and may often be observed on green fruit at the time of cargo discharge in the United Kingdom. This form of fruit-rot has been referred to as 'non-latent anthracnose' (Meredith, 1960) in order to distinguish it from a similar rot which develops at a later stage of maturity following infection of unwounded tissues ('latent infection', Simmonds, 1941; Chakravarty, 1957).

In this paper some of the factors affecting the development of wound-infections are described: also reported are the results of commercial-scale trials designed to test the effectiveness of the antifungal antibiotic nystatin in controlling disease.

Materials and methods

Unless stated otherwise, experiments were carried out on harvested bananas of the Lacatan variety. Inoculations were performed by scratching the skin with a needle-point to produce a wound approximately 2 mm. in diameter and 0·5 mm. deep. The wounded area was then sprayed with a water suspension of conidia of *G. musarum*, adjusted to the desired concentration by means of a haemocytometer.

Fruit was stored and ripened under the conditions described previously (Meredith, 1960). During the cold-storage period, which corresponds with the ocean transportation period, fruit was enclosed in perforated 'Polythene' tubing; however, to conform with the practice often adopted under commercial conditions, the covers were removed at the commencement of the ripening process.

The results of inoculation were recorded when the fruit was fully mature, i.e. having an even yellow skin-colour flecked with brown 'maturity' spots. Records were made of:

(1) *Disease incidence*. This was the number of lesions developed at inoculated points, expressed as a percentage of the total number of inoculations.

(2) *Disease severity*. This was assessed on the basis of lesion length.

The following lesion rating was employed:

Description	Rating
No infection	0
Lesion up to 12·5 mm. long	1
Lesion 12·5–25·0 mm. long	2
Lesion 25·0–37·5 mm. long	3
Lesion >37·5 mm. long	4

The lesion rating scores for each replicate series of inoculations were totalled for purposes of statistical analysis.

Experimental

Inoculum concentration

The effect of varying inoculum concentration on disease incidence and severity was tested. A stock suspension of conidia (S), containing approximately 3×10^4 spores ml., was prepared from abundantly sporing agar cultures of *G. musarum*: successive tenfold dilutions were made with distilled water. From each of ten newly harvested bunches, six hands were detached. On each hand ten fingers were wounded and then sprayed with approximately 1 ml. inoculum suspension.

Table 1. *Effect of inoculum concentration on the incidence and severity of anthracnose*

	Inoculum concentration				
	S	S/10	S/100	S/1000	S/10,000
Percentage successful inoculations	99	94	14	6	7
Disease severity	284	223	17	7	8

A total of 100 inoculations was performed with each inoculum concentration. The inoculated fruit was stored in boxes for 10 days at 55° F. and then ripened at 70° F. Results are set out in Table 1, in which the severity data are total lesion rating scores out of a possible total of 400.

The effect of decreasing inoculum concentration is evident. The magnitude of the effect probably varies according to the particular strain of *G. musarum* used

(Ashby, 1931), the source (agar cultures *v.* infected banana material) and to the age of conidia. Nevertheless, the results were helpful in choosing the range of concentrations over which a reasonable response may be expected. Hence in subsequent experiments, spore suspensions containing 3×10^3 to 3×10^4 spores/ml. were used for inoculation purposes.

Size of stem and position of hand

In relation to experimental design, it was considered important to investigate possible effects on anthracnose development of (1) the size of stem as indicated by the number of hands, e.g. 6, 7, 8 or 9, and (2) the relative position of the hand on the main stalk, e.g. large uppermost hand (butt-end), intermediate hands or small lowermost hand (tip-end). The four above-mentioned stem sizes, replicated eight times, were selected and ten fingers on each of the butt-end, tip-end and one of the intermediate hands were inoculated with *G. musarum*. Disease assessment was carried out after the fruit had been stored for 10 days at 55° F. and for a further 6 days at 70° F., by which time it was fully mature. The results indicated that small hands were more severely diseased than large ones, but the differences did not approach the significant level of probability.

Stem size would appear to be one indicator of plant vigour, for in any uniformly treated field, the tallest and most robust plants commonly yield the largest stems of fruit (Lumsden, 1959). However, the results obtained here suggested that plant vigour had no significant effect on disease incidence or severity.

The results of this trial indicated that it was not essential to use stems of equal size for experimentation. However, in order to minimize possible errors caused by positional effects within a stem, hands to be inoculated were chosen randomly.

Maturity of fruit

In the field the degree of maturity of unreaped fruit is assessed by the 'fullness' of individual fingers. Within the banana industry the following 'grades' of maturity of green fruit are distinguished:

'Thin'—flattened sides, extremely prominent ridges.

'Three-quarters'—slightly rounded sides, prominent ridges.

'Light full three-quarters'—medium rounded sides, less pronounced ridges.

'Full three-quarters'—well-rounded sides, slight ridges.

'Full'—round or nearly round in cross-section, ridges hardly evident.

Experiments were set up to compare the development of anthracnose on 'thin', 'light full three-quarters' and 'full' fruit. On each of six replicate stems of each grade, thirty fingers were wound-inoculated with *G. musarum*. The fruit was stored for 11 days at 55° F. and then for 6 days at 68–70° F., when it was fully ripe. Results obtained in two independent experiments are summarized in Table 2, where disease severity scores are out of a maximum possible of 120.

The results of both experiments suggest that the degree of maturity of green fruit had a marked effect on anthracnose development, 'full' fruit being more severely

diseased than 'thin' fruit. There was no significant effect on disease incidence. These experiments emphasized the importance of selecting fruit of uniform maturity when investigating other factors.

TABLE 2. *Effect of maturity of bananas on the development of anthracnose following wound-inoculation with* Gloeosporium musarum

	Exp. 1		Exp. 2	
Maturity ('grade')	Mean disease incidence	Mean disease severity	Mean disease incidence	Mean disease severity
'Thin'	91·6	41·3	98·0	52·8
'Light full three-quarters'	92·7	54·2*	98·0	66·6**
'Full'	95·8	64·0***	100	78·0***
S.E.	—	±5·24	—	±3·54

* Significantly different from mean for 'thin' fruit at 5 % level of probability: ** at 1 % level: *** at 0·1 % level.

Varietal susceptibility

Agati (1922) found that sweeter varieties, including Lacatan, were more susceptible to infection by *G. musarum* than less sweet ones such as plantains. Most of the fruit now exported from Jamaica comprises the Lacatan variety, but a relatively small number of stems of Robusta and Gros Michel fruit are also shipped. Trade reports describing the condition of Jamaican bananas at the time of arrival, and after ripening, in England were studied to obtain information about the severity of anthracnose on the various varieties. Although these reports were essentially qualitative, they were consistent in recording disease as negligible (0), trace (1), slight (2), medium (3) or heavy (4). The numbers in parentheses were applied here as a disease rating. Statistical analysis of the ratings obtained from 235 separate consignments of fruit indicated that the amount of anthracnose on Gros Michel was significantly less ($P < 0{\cdot}001$) than that on Lacatan. Experiments were initiated in an attempt to account for this.

In two experiments, the development of anthracnose on fruit of the Lacatan, Robusta and Gros Michel varieties was compared. For each variety, 'light full three-quarters' to 'full three-quarters' fruit was selected. A total of 175 wound-inoculations, evenly distributed among seven replicate stems, were performed on each variety. The results obtained after cold-storage and ripening are given in Table 3; scores for severity are out of a maximum possible of 100.

The results for disease incidence and severity suggest that Gros Michel fruit is less susceptible to wound-infection than either the Lacatan or the Robusta variety, thus helping to explain the data obtained from trade reports. At the end of the cool-storage period, lesions on Gros Michel were inconspicuous, unlike those on Lacatan or Robusta which were often well developed. Only after transfer to the higher temperature was there any appreciable size increase on the first-mentioned variety. It is well known within the banana industry that Gros Michel fruit ripens more rapidly than Lacatan or Robusta. This occurred in the present experiments,

so that at the time of recording, Gros Michel fruit was at a more advanced stage of maturity than the other varieties. However, it is evident that this maturity factor did not nullify the effect of differing susceptibility. There was no significant difference between the data for Lacatan and Robusta fruit; this was not unexpected for it is believed that these varieties are more closely related to each other than they are to Gros Michel (Simmonds, 1959).

TABLE 3. *Development of anthracnose on three varieties of bananas after wound-inoculation with* Gloeosporium musarum

	Exp. 1		Exp. 2	
Variety	Mean disease incidence†	Mean disease severity	Mean disease incidence†	Mean disease severity
Gros Michel	50·2°	27·3	60·8°	32·1
Lacatan	63·4°**	41·3*	82·0°***	59·1***
Robusta	66·3°***	47·3**	84·3°***	64·8***
S.E.	±3·70°	±5·44	±3·89°	±4·62

† Angular transformations of mean percentage numbers of lesions developed.
* Significantly different from Gros Michel mean at 5 % level of probability; ** at 1 % level; *** at 0·1 % level.

Bagging of fruit during cold storage and ripening periods

Under present conditions in Jamaica, each stem of exported fruit is enclosed in a perforated 'Polythene' bag prior to stowage in refrigerated ships. This measure is designed to reduce the amount of mechanical injury to the fruit both before and during ocean transportation. At most ripening stores in the United Kingdom, bags are removed at the commencement of the ripening process; however, a few stores retain bags in the belief that ripening behaviour is improved. An experiment was set up to assess possible effects of bagging on the development of anthracnose during cold storage and ripening. A 2^2 factorial arrangement, with five replicate stems for each treatment combination, was employed: twenty-five fingers were inoculated on each stem. The treatment combinations were:

(1) Cold storage and ripening carried out with bag.
(2) Cold storage with bag, ripened without bag.
(3) Cold storage without bag, ripened with bag.
(4) Cold storage and ripening carried out without bag.

Immediately after inoculation, fruit was transferred to the cold-storage chamber and maintained at 55° F. for 11 days prior to ripening at a higher temperature (68° F.). Mean scores for disease severity (out of a maximum possible of 125) are given in Table 4.

Disease incidence is not recorded here since there were no marked differences between the variously treated fruit. The presence of bags during cold storage resulted in a significant increase of disease severity relative to that on unbagged fruit, probably because of more favourable humidity conditions at the surface of bagged fruit. However, it is doubtful whether the magnitude of the effect produced

would be of great practical importance. Also, under shipboard conditions there is reason to believe that the presence of bags does not cause a marked increase of humidity at the fruit surface, for even without bags the surface of fruit is often covered by a film of moisture (Messrs Elders and Fyffes Ltd.—personal communication).

TABLE 4. *Effect of 'polythene' bags on the severity of anthracnose*

	Ripened		
Cold storage:	With bag	Without bag	Mean
With bag	66·4	69·2	67·8
Without bag	59·6	52·8	56·2
Mean	63·0	61·0	

Summary analysis of variance

Source of variation	Degrees of freedom	Variance
Bag/cold storage	1	672·80*
Bag/ripening	1	20·00
Interaction	1	115·20
Error	16	82·62

* Significant at 5 % level of probability.

During ripening, the presence of bags had no significant effect on development of rot. By this time it is likely that the fungus had penetrated tissues to such an extent that further growth was independent of external humidity.

Effect of temperature and humidity on early stages of infection

After harvest, a large proportion of Jamaican export fruit has to be transported by road or railway to the loading wharves. Partly because of this, and also because fruit may remain on the wharf for several hours before loading, the interval between reaping and loading is seldom less than 12 hr.; in extreme cases it may approach 40 hr. It is evident, therefore, that infections by *G. musarum* which are established shortly after reaping may develop for some time under tropical conditions. As a result of studies on time and temperature factors, Wardlaw & McGuire (1932) recommended (1) the minimum of delay between reaping and loading, and (2) rapid cooling of cargoes after stowage. In this way they were able to reduce considerably the extent of fruit-rot caused by several fungi.

Experiments were set up to investigate the effect on anthracnose development of various periods of exposure to tropical temperatures prior to the commencement of refrigeration. In the first experiment, 'light full three-quarters' fruit was inoculated at intervals of 72, 48, 24, 12 hr. or immediately before transfer to the cool chamber. During the interim period it was stored in the open while wrapped in perforated 'Polythene' tubing. Rapid cooling was achieved by transferring the fruit to a room maintained at 55° F. After 11 days cold-storage, ripening was carried out at 70° F. The results of inoculation are shown in Table 5,

where values for disease incidence and severity are means of five replicate stems, each bearing twenty-five inoculated fingers. The results indicate that the amount of rotting decreased with increasing storage intervals under tropical conditions. After about 48 hr. it was observed that nearly all inoculations were dry, and it was thought likely that humidity had limited fungal growth, particularly in the 72 hr. series. This possibility was investigated in the next experiment.

TABLE 5. *Effect on anthracnose of different periods of exposure to tropical temperatures prior to cold storage and subsequent ripening*

	Duration of exposure to tropical temperatures (hr.)				
	0	12	24	48	72
Mean disease incidence	99·2	94·4	86·4	84·0	49·6
Mean disease severity	61·0	56·0	47·8	45·4	22·4

On each of twenty-four stems, twelve fingers were inoculated with *G. musarum*. The stems were assorted randomly into three groups of 8, which were then treated in one of the following ways:

(1) Enclosed in unperforated 'Polythene' bags.
(2) Enclosed in perforated 'Polythene' bags.
(3) Not enclosed.

All fruit was stored under prevailing field conditions and, at chosen intervals, successive lots of twelve fingers per stem were inoculated. After the final inocula-

TABLE 6. *Effect of humidity and various intervals at tropical temperatures on anthracnose development*

		Hours at tropical temperature (76–87° F.)				
Treatment	R.H.(%)	0	12	24	48	72
		Mean disease incidence				
Closed bag	92–100	95·0	90·8	73·3	75·0	61·7
Perforated bag	87–90	90·0	83·3	67·5	48·3	24·2
No bag	61–87	94·2	87·5	61·7	46·7	31·7
		Mean disease severity				
Closed bag	As above	25·9	25·4	17·9	21·4	14·1
Perforated bag	As above	26·9	23·1	16·9	11·6	5·4
No bag	As above	27·6	25·1	15·0	12·9	5·9

Summary analysis of variance

		Variance	
Source of variation	Degrees of freedom	Disease incidence†	Disease severity
Time/temperature	4	7069·3***	1351·38***
Bagging	2	1484·82***	189·51**
Interaction	8	314·59	63·55
Error	105	157·78	48·73

† Analysis performed on angular transformations of percentages.
** Significant at 1 % level of probability; *** at 0·1 % level of probability.

tions (0-hr. series), stems were enclosed in perforated 'Polythene' bags and placed in the cold store (55° F.) where they remained for 12 days. Records were taken after a further 7 days storage at 70° F. when the fruit was fully mature. The results are set out in Table 6, together with data for relative humidity at the surface of the variously treated fruit.

After about 24 hr. field storage, disease incidence and severity showed a marked decrease and continued to decrease with increasing storage intervals. Probably because of more favourable humidity conditions this effect was least pronounced on fruit enclosed in unperforated bags, thus accounting for the significant variance ratio for bagging treatment/error. There were no significant differences between the data for fruit wrapped in perforated bags and those for unwrapped fruit. For most of the 72 hr. period, the surface of fruit contained in unperforated bags was covered by a film of moisture. It seems unlikely, therefore, that limiting humidity caused the decline in infection. A satisfactory explanation of this decrease cannot be offered here, but it is possible that prolonged exposure to tropical temperatures had some effect on host resistance (Garrett, 1959).

In conclusion, there is little reason to believe that an interval of 40 hr., or even longer, between reaping and loading will result in an increase of anthracnose relative to that on fruit loaded with less delay.

Seasonal variation

Trade reports compiled in the United Kingdom were studied to determine whether the amount of anthracnose varied at different times of the year. For each month of the period studied, a disease index, based on the arbitrary rating described on p. 521, was calculated.

Anthracnose was most evident during the autumn and winter of each year, becoming less conspicuous in spring and early summer. An explanation for this variation must await further investigation, but two possible factors affecting it are (1) seasonal abundance of inoculum, and (2) varying susceptibility of fruit to mechanical injury at different times of the year. Both factors are now being studied. There is no evidence that the amount of mechanical injury caused by rough handling varies from season to season.

Chemical control

In a previous paper (Meredith, 1960) it was reported that the antifungal antibiotic nystatin (Brown & Hazen, 1957), applied in a post-inoculation fruit-dip, effected good control of infection by *G. musarum*. It was considered desirable, therefore, to test the compound under commercial conditions: these experiments are described below.

At the banana-loading wharf, a dipping tank was charged with 400 gallons of water. Nystatin, in the form of 'Mycostatin 20' (agricultural grade of nystatin), was added at the rate of 400, 300 or 200 p.p.m. respectively in successive trials. Also, approximately 0·1 % of a wetter of the secondary alcohol sulphate type was incorporated in the dipping medium. It is current practice to wash fruit for

export in dilute sodium bisulphate and then in water in order to remove deposits of Bordeaux mixture. As each truckload of fruit arrived from the plantations, it was divided randomly into two equal groups of stems. One lot was washed in acid and water and then dipped for 1–3 min. in the nystatin dip, which was agitated constantly to keep the compound in suspension. The remainder, serving as controls, were washed in acid and water only. All fruit was then wrapped in perforated 'Polythene' bags and stored on the quayside until it was loaded in the refrigerated hold of the ship (4–14 hr.).

Disease assessments were made in the United Kingdom when the fruit was fully mature. A disease index number, based on the rating given on p. 521, was calculated as follows:

$$\text{Disease index no.} = \Sigma\,\% \textit{ number of stems in category} \times \textit{category rating}/4$$

Data obtained from different shipments and at various banana-ripening stores are given in Table 7.

TABLE 7. *Effect of nystatin on the severity of anthracnose: disease index numbers obtained from successive shipments of fruit*

Date of shipment	Treatment	Total no. stems	Ripening store no. 1	2	3	4	5	6	7	8	9	Mean
15. ix. 59	Nystatin (400 p.p.m.)	994	2·4	38·6	11·2	39·1	9·3	2·3	1·7	—	—	14·9
	Controls	1124	36·9	11·5	56·3	43·6	29·8	6·8	3·7	—	—	26·9
23. ix. 59	Nystatin (300 p.p.m.)	1279	18·3	23·5	8·7	0	20·2	9·8	2·6	5·5	22·9	12·4
	Controls	1566	24·3	26·9	12·1	9·8	56·8	36·8	39·0	11·1	23·7	26·7
29. ix. 59	Nystatin (200 p.p.m.)	910	23·2	11·8	0·8	0·3	12·3	1·0	4·1	—	—	7·6
	Controls	1151	19·3	43·5	22·9	57·3	45·0	1·4	13·6	—	—	29·0
5. x. 59	Nystatin (400 p.p.m.)	1208	Not recorded									
	Controls	1131	Not recorded									
14. x. 59	Nystatin (300 p.p.m.)	1282	3·1	3·2	32·5	22·8	7·3	22·9	1·8	11·9	14·7	13·4
	Controls	1113	16·6	12·6	52·1	37·4	8·5	32·0	8·2	20·0	17·6	22·8
21. x. 59	Nystatin (200 p.p.m.)	1384	28·7	18·6	15·4	22·7	10·5	22·6	2·1	3·0	5·6	14·3
	Controls	1666	58·4	28·5	19·9	28·1	17·6	50·6	21·6	4·8	9·4	26·5

Although there was considerable variation between data obtained from different ripening stores, the data suggest that nystatin brought about some control of anthracnose: comparison of means from successive shipments indicates a range of control in the order of 40–70%. There was no evidence that 400 p.p.m. of nystatin was more effective than the lower concentrations tested, thus substantiating earlier experimental findings (Meredith, 1960). In earlier experiments, it was noted that at a concentration of 400 p.p.m. nystatin caused a slight reduction in the rate at which fruit ripened. No such effect was observed under commercial ripening conditions.

Discussion

Perhaps the most important factor governing the amount of wastage of bananas due to anthracnose is the care with which handling is carried out before and during loading into ships' holds. Wardlaw & McGuire (1931, 1932) and Wardlaw (1931) have emphasized the importance of careful handling at this stage to reduce wastage caused by fungi which gain access to the tissues through mechanically induced wounds. Recent investigations in Jamaica by Leach (1958, 1959) have confirmed this. On the other hand, there is evidence to suggest that a negligible proportion of anthracnose results from infection after ocean transport, i.e. during discharge and transport in the United Kingdom (Meredith, 1959).

Experiments described in this paper suggest that several other factors may affect the development of anthracnose. It was found that 'full' fruit was more severely diseased than 'thin' fruit. In Jamaica, growers are instructed to supply 'full' Lacatan bananas for export purposes. By reaping fruit at a slightly earlier stage of maturity it may be possible to reduce the amount of wastage caused by anthracnose. Such a measure might be of particular importance if in future, as seems probable, fruit is bought according to weight and not size (e.g. 6, 7, 8, 9 hands) of stem: there may be a tendency to delay reaping until fruit is 'over full'.

Although Gros Michel appeared to be less susceptible to anthracnose than Lacatan or Robusta, there is at present little practical value in the finding. The former variety is highly susceptible to *Fusarium* wilt and for this reason is not planted extensively in Jamaica (Rishbeth, 1955).

Chakravarty (1957), working with Gros Michel or Cavendish bananas, found that expressed juice of green banana peel had an inhibitory effect on germination of conidia: juice from yellow skin stimulated germination. It was suggested that this inhibitory effect may be due to the presence of tannin, the concentration of which decreases as the fruit ripens. It is possible that there is a similar chemical basis of differing varietal susceptibility.

Wardlaw (1931) reported that blemishes caused by *G. musarum* did not develop appreciably until the fruit was almost full ripe. In contrast, Meredith (1960), working with Lacatan bananas, observed well-developed lesions on immature, green fruit following wound-inoculation and storage for 10–12 days at 55° F. However, in the current investigation, it was found that on similarly treated Gros Michel, lesions did not become conspicuous until after transfer to the ripening room. Much of Wardlaw's work appears to have been carried out on Gros Michel fruit and this may help to explain the contrasting observations.

The evidence from commercial-scale experiments suggests that a post-harvest dipping treatment with nystatin afforded some measure of control of anthracnose. This confirms the results of similar trials conducted by Castellani, Beccari & Matta (1958). However, control was not as good as in laboratory experiments (Meredith, 1960). In the latter, it was found that percentage control was inversely related to the period of incubation between inoculation and treatment: little control was evident with periods exceeding about 30 hr. In the shipment trials, much of

the fruit was reaped nearly 20 hr. before being dipped. Infections that were established shortly after harvest may have escaped antibiotic action, possibly explaining some instances of poor control. It would seem desirable to minimize the delay between reaping and nystatin treatment.

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ADDENDUM

Experiments carried out subsequent to those described in this paper and earlier (Meredith, 1960) have shown that nystatin does not control finger-stalk rot ('Santa Marta stem-end rot') caused by *G. musarum*. Details of this work will be published elsewhere.

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STUDIES IN THE BACTERIAL DISEASES OF SUDAN CROPS

VI. THE PRODUCTION OF ATYPICAL SYMPTOMS BY *XANTHOMONAS MALVACEARUM* (E. F. Sm.) DOWSON AND OTHER *XANTHOMONAS* SPP.

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(With Plate 5)

The atypical symptoms first described by Bryan (1932) of the angular leaf spot disease of cotton caused by *Xanthomonas malvacearum* (E. F. Sm.) Dowson were reproduced by inoculation into seeds, stem apices or buds. The lesions that developed on the veins of the newly produced leaves were elongated and water-soaked, becoming dark brown. The leaf tissue dependent upon infected veins became yellow, flaccid and withered. The development of these symptoms was enhanced when inoculations were made into opening buds or germinating seeds as compared with inoculations into closed buds or dormant seeds.

In other bacterial diseases caused by *Xanthomonas* spp., somewhat atypical symptoms could also be produced by bud inoculation into the appropriate host. Those produced by *X. ricini* (Yoshi & Takimato) Dowson on castor, closely resembled the vein lesions described above on cotton but resulted only from bud inoculations; inoculations into stem apices and seeds failed to produce them. In dolichos bean inoculated with *X. phaseoli* (Smith) Dowson, atypical symptoms were produced only by seed inoculations and were confined to the first simple leaves (prophylls).

The differences in the production of atypical symptoms on the three hosts are correlated with differences in host structure and with the degree of virulence of the pathogen. The leaf parasite *X. ricini*, for example, which cannot infect castor bean stems, does not produce atypical symptoms when inoculated below the stem apex.

From the data discussed below, the incidence of atypical symptoms is attributed to infection either of an actively growing tissue or of a telescoped structure which subsequently completes its development.

The atypical symptoms of the cotton disease are not caused by a special strain of *X. malvacearum*. Further, they are not a peculiarity of this disease but may also develop in other necrotic diseases under similar conditions.

Introduction

The first report of the production of atypical symptoms by *Xanthomonas malvacearum* (E. F. Sm.) Dowson was made by Bryan (1932). These symptoms have been recently fully described and investigated by Thiers & Blank (1951), by Wickens (1956) and by Last (1958). Bryan (1932) suggested that they were caused by a particular strain of *X. malvacearum* having minor cultural differences from the

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common strain of the pathogen. She suggested that the particular strain infected the vascular tissue; hence the term 'vascular infection' has since been adopted for this symptom. Thiers & Blank (1951) noted that in some cases of severe stem infection the pith, the vascular rays and the phloem, as well as the cells of the primary xylem, were attacked and damaged. Occasionally, the bacteria were also observed in the large vessels of the xylem, but no apparent damage had been done.

Wickens (1956) reproduced atypical symptoms on cotton seedlings by presoaking the seeds in a water suspension of the pathogen. The cotyledons developed atypical, dull-green flaccid areas extending from the periphery, followed by vascular discoloration in the petioles of cotyledons, in the hypocotyl and in parts above the cotyledonary node. On one or more of the true leaves similar flaccid areas might appear on the lamina. Wickens suggested that these symptoms were typical of a vascular disease.

Last (1958) reproduced similar effects on young cotton plants by artificial inoculations (piercing) into the stems just below the apex. He stated that the probability of the bacteria moving within the plant increased the nearer the point of inoculation was to the apex. Last (1959), by inoculations into main veins, noted that the bacteria spread more rapidly in the veins of younger leaves than of older ones. Both Wickens and Last suggested that the bacteria migrate from the stem to leaf veins and vice versa in the discoloured vascular tissue. They imply that the xylem vessels are the channels of spread. They were unable to isolate the pathogen beyond the vein lesions, and Last (1958) stated that *X. malvacearum* was never detected more than 0·8 cm. from obvious external symptoms. Neither Wickens nor Last investigated the histology of the symptoms they describe. Thiers & Blank, however, could find no evidence that the bacteria actually travelled in the xylem.

This paper is a contribution towards a clearer understanding of the production of the atypical symptoms of the blackarm disease. Cotton breeders, at the Cotton Breeding Section, Shambat, feared that these symptoms might be caused by a new strain of *X. malvacearum* which could interfere with their breeding programme. The investigation also deals with the production of atypical symptoms by other species of *Xanthomonas*, e.g. *X. phaseoli* and *X. ricini*, on their respective hosts.

Anatomical studies

Materials for anatomical studies were fixed in formalin–acetic–alcohol, dehydrated in ethyl on tertiary butyl alcohol and embedded in paraffin wax. Sections were cut 10–15 μ thick and stained with safranin and light green in cellosolve or in safranin in 50% alcohol and fast green in clove oil.

The bacteria in the leaf spots on Domains Sakel (susceptible) were found in masses in the collapsed mesophyll. The spots were limited by the vascular sheaths of relatively large veins. Wood vessels and wood parenchyma were not invaded but some of them were filled with dark-staining material. In the more resistant types, the spots were considerably smaller, limited by smaller veins, and the masses of bacteria were generally less dense compared with those in the susceptible cotton.

In both suceptible and resistant types, the bacteria were confined by the vascular sheath.

The bacteria in severe infections of Domains Sakel were found in the veins and adjacent mesophyll tissue. The lesions were laterally bordered by vascular sheaths of minor veins or by vascular bundles in the mesophyll. The affected tissue appeared as a disorganized mass of host cells and bacteria. The vessels were not, as a rule, invaded except at an advanced stage when a few wood vessels were occupied by bacteria, but some wood vessels and parenchyma cells became filled with a dark staining substance. In milder infection, bacterial cavities were found in the intercellular spaces of the developing vascular sheath. The vein cells remained intact and a few were darkly stained. The wood vessels in mild infections were free of bacteria, but a few were filled with a dark staining substance.

Transverse sections of swollen axillary buds of both cotton and castor showed close resemblance in the structure and arrangement of leaf primordia. There are usually three to four embryonic leaves in the bud showing different stages of development. The leaf primordium is round and collar-like at first. The palmately arranged veins are distinct at an early stage, the median vein being followed by two laterals. The basal lateral pair of veins appear later on the incurved margins of the primordium. There is little lateral growth of the leaf lobes in the bud. When the bud opens, the main veins appear as definite ridges on the abaxial surface of the plicate leaf and are very thick in proportion to the rest of the lamina. The ground tissue of the lamina appears as narrow furrows. The vascular sheath which surrounds the vascular elements of the veins of the mature leaves is absent and in its place loose, thin-walled cells are found.

Isolation of *Xanthomonas malvacearum*

Eleven isolations of *Xanthomonas malvacearum* were made from infected Domains Sakel cotton grown at Shambat as follows: (i) three from typical angular leaf spots, (ii) four from infected veins (atypical symptoms), (iii) four from stem lesions (blackarm). The organism was readily isolated from affected parts surface-sterilized 1 min. in 30% hydrogen peroxide. No trace of the pathogen was detected more than 1–3 cm. distant from visible lesions.

The isolates were compared by the usual procedures (Dowson, 1957) and all agreed in their morphological, cultural and biochemical characters, thus indicating that the production of atypical symptoms is not associated with the existence of separate strains of the pathogen.

Inoculation into cotton

Unless otherwise stated, inoculations were carried out after the end of the rainy season, i.e. from October to April, on plants protected against insects by frequent dusting with DDT. Potted plants of Domains Sakel were inoculated from 2- to 3-day-old cultures of *X. malvacearum* grown on yeast extract–glucose agar. Control inoculations using sterile water gave no infections.

Leaf inoculations

Expanding cotton leaves, 5–10 days old, and fully expanded mature leaves, 20–30 days old, were inoculated either (i) by inserting stainless 'thinnest' entomological pins previously submerged in the bacterial suspension into one of the main veins of the leaf, or (ii) by spraying the bacterial suspension on to the lower surface. Isolates from (*a*) natural vein lesions, (*b*) stem lesions, or (*c*) angular leaf spots, produced similar symptoms on cotton leaves; 138 inoculations were made into veins of young leaves and seventy-eight into veins of mature leaves. In the former the resulting vein lesions were first water-soaked and dark-green, turning brown later, and extending to 2–4 cm. in 4–5 weeks. Many lesions ran back into the petioles, killing the whole leaf and often the axillary bud. Some extended laterally into secondary veins and a few spread distally; occasionally they spread from the veins into narrow strips of the adjacent mesophyll. Partial or complete chlorosis occurred, associated sometimes with inrolling of the leaf lobe. When veins of mature leaves were inoculated, only small, oval to slightly elongated lesions developed.

Young expanding leaves sprayed with bacterial suspension each developed twenty to sixty angular spots, 2–4 mm. diam.: inoculated mature leaves developed approximately 200 spots, 1–2·5 mm. diam. In the young leaves, nineteen vein lesions developed, but none in the mature leaves.

Stem inoculations

An isolate of *X. malvacearum* obtained from vein lesions was inoculated as described above into the stems of 2-month-old cotton plants either (i) 2–5 mm. below the apex, or (ii) just above the scars of the cotyledons, i.e. into the woody part 5–7 cm. above soil level. The pins were left in and the plants were kept under observation for 3 months.

Table 1. *Summary of results of inoculations made below stem apices*

Order of leaf production after inoculation	Total leaves produced	No. of infections on		
		Leaves	Petioles	Veins
1st	20	17	16	5
2nd	16*	16	13	5
3rd	7	6	3	4
4th	2	1	1	1

* 1st leaf was not infected in two of these plants.

At the end of the first month the apices of the plants inoculated below the stem apex had grown 1·8–5·1 cm. beyond the point of inoculation and formed two to four expanding leaves. Watersoaked stem lesions developed in all the twenty plants of this batch, keeping pace with the apex but also extending 2–3 cm. below points of inoculation. On the leaves, no angular leaf-spots appeared, only vein and petiole

infections. The latter developed on most of the leaves produced above points of inoculation, whereas up to six vein infections appeared suddenly on a few leaves, not necessarily those with infected petioles or those nearest to points of infection. No vein infection appeared below the stem lesions but the petioles of three leaves situated within the downward extension of the lesion were infected. The ratio of infected petioles to the total number of infected leaves decreased from the first to the third leaf parallel to an increase of the ratio of the leaves showing vein lesions to the total number infected (Table 1).

During the following 2 months, no new vein infections appeared. The parts of the stems above the points of infection, including the apices, died out. On the stems inoculated immediately above the cotyledonary scars, only a local blackening of the tissue around the point of inoculation was observed.

Bud inoculations

Ten, 2- to 3-month-old potted plants were topped by cutting off the green part of the stem and removing all the leaves on the woody parts. Three days later, the dormant buds on the woody stems began to flush. Single drops of a thick bacterial suspension were deposited on five buds per plant with a long pin, using its head as a carrier. The plants were covered with glass bell-jars for 2 days.

Forty-four buds were inoculated of which forty-one produced new shoots. Two shoots showed stem lesions and eight showed vein infection on the first, second or third leaves. No petioles were infected and no angular leaf-spots were produced.

In another experiment, two series of eight plants each were inoculated. In the first series, the plants were topped and all leaves removed 4 days before inoculation, whereas, in the second lot, the plants received this treatment on the day of inoculation. The buds were inoculated by spraying on a bacterial suspension.

Of the thirty-five buds inoculated in the first series (open buds), twenty-five developed into lateral shoots. Eight of these were severely infected immediately after the production of the third leaf during the third week after inoculation and died. Of the remaining seventeen shoots, three developed stem lesions independently from petiole lesions.

In the second lot, of the thirty-three dormant buds inoculated, twenty-two produced new shoots. Five were blighted and died during development after producing two or three leaves. Three of the remaining fifteen shoots developed stem lesions independently from leaf infection. Otherwise, infection appeared on the veins and petioles as shown in Table 2. These results showed similar trends in that: (i) stem, petiole and vein infections were produced independently on resulting shoots; (ii) leaf infection gradually decreased higher up the shoots and ceased to appear above a certain point; and (iii) petiole infection ceased to appear on the leaves produced at lower nodes than vein infection. Infection in the two series differed, on the other hand, in that: (i) infection in closed buds was, generally, appreciably milder than in open buds, not only in the number of infected leaves (Table 2) but also in the number of infected veins per leaf and the extent of lesions (Pl. 5, fig. 2); (ii) the second leaf on the shoots resulting from inoculated open buds

and the first leaf on the shoots from closed buds marked the points of maximum infection, whereas the fifth leaf in the first instance and the fourth in the second marked the points of maximum upwards extension, and (iii) two first leaves developing from open buds showed a few large angular spots while those developing from closed buds showed no angular spots.

In another experiment nine potted Domains Sakel plants, about 2 months old, were topped as before, and 4 days later a single open bud, situated approximately halfway along the stem, was inoculated after protecting the stem below it with greased paper.

Of the nine inoculated buds, one remained dormant, three produced small flushes that were blighted and died, and five developed into shoots showing vein and petiole infections. Thirteen shoots were produced above, and four shoots below, the inoculated buds. None of these shoots showed any sign of infection within 6 weeks after inoculation.

Table 2. *Petiole and vein infections resulting from bud inoculations*

Order of leaf production after inoculation	Infection on shoots resulting from inoculated buds* Open buds			Closed buds		
	Total infected	Petiole	Vein	Total infected	Petiole	Vein
1st	6 out of 17	1	6	8 out of 15	3	5
2nd	12 out of 17	5	9	6 out of 15	3	3
3rd	7 out of 17	1	7	6 out of 15	2	5
4th	5 out of 15	1	4	1 out of 15	0	1
5th	3 out of 15	0	3	0 out of 11	0	0

* The figures in the table indicate the number of leaves showing lesions developing directly on the petioles and veins about 4 weeks after inoculation. The spread of infection from veins to petioles or vice versa is not included.

Seed inoculations

Domains Sakel seeds were surface-sterilized in 30% H_2O_2 for 3 min. Inoculations were made: (i) into germinating seeds kept in a moist chamber for 48 hr., or (ii) into dormant seeds. The seeds were soaked in a watery suspension of *X. malvacearum* for 4 hr. and were then sown, together with controls, in equal parts of fine sand and Nile silt in four tin trays, twenty seeds per tray, at a depth of about 5 cm.

The cotyledons subsequently showed two types of infection: (i) relatively large, roundish or slightly angular spots, and (ii) vein lesions surrounded by yellow flaccid areas on a few cotyledons. On the true leaves, vein and petiole lesions developed but no spots were observed. In many, infection extended to the hypocotyls, causing black-arm.

Table 3 shows that more infection occurred when the germinating seeds were inoculated. The prevalence of cotyledonary spots suggested early infection, probably by soil contact. The foliage leaves from the first to the third above the cotyledons were not infected in seedlings from seeds inoculated before germination. The development of vein lesions on the true leaves of germinating seeds seemed to be dependent upon the stage at which infection first appeared on the stem (Pl. 5,

fig. 1). Thus where the stem was infected before elongation of the first internode above the hypocotyl, the apex was killed (six out of thirty showing stem infection). Where the stem was infected at the time the first internode was elongating and the stem apex not more than 1·5 cm. above the cotyledons, the growth seemed to be checked and the first and second leaves showed vein lesions only (three out of thirty). Where the stem was infected after the first internode had reached its maximum length and the apex 3–5 cm. above the cotyledons, stem lesions were confined to the area adjacent to the junction of stem and cotyledons. The leaves above did not show any sign of infection.

TABLE 3. *Infection on seedlings from inoculated seeds*

	Infections from inoculated seeds	
	Germinating seeds	Dormant seeds
Total infected ...	31 out of 48	21 out of 57
Cotyledons (spotting)	56	25
Cotyledons (vein lesions)	9	5
1st leaf (vein lesions)	6	0
2nd leaf (vein lesions)	3	0
3rd leaf (vein lesions)	1	0
Stem	30	1

INOCULATION INTO OTHER HOSTS

In a preliminary inoculation experiment 8–14 plants of each of dolichos bean (*Dolichos lablab* L.), haricot bean (*Phaseolus vulgaris* L.), catjang cowpea (*Vigna unguiculata* (L.) Walp), soybean (*Glycine soja* Sieb. & Zucc.), lucerne (*Medicago sativa* L.), jute (*Corchorus olitorius* L.), castor (*Ricinus communis* L.) and cotton (*Gossypium barbadense* L.) were inoculated with recent isolates of *X. phaseoli* (Smith) Dowson, *X. phaseoli* v. *fuscans* (Hedges) Starr & Burkholder, *X. phaseoli* f. sp. *vignicola* (Burkholder) Sabet, *X. phaseoli* f. sp. *sojense* (Hedges) Sabet, *X. phaseoli* f. sp. *alfalfae* (Riker, Jones & Davis) Sabet, *X. nakatae* var. *olitorii* Sabet, *X. ricini* (Yoshi & Takimoto) Dowson (Sudanese strain) and *X. malvacearum* (E. F. Sm.) Dowson respectively. The plants were topped and all but three of the leaves were removed. Three days later, the plants were sprayed with a watery suspension of the respective pathogens and covered with glass bell-jars for 48 hr.

Normal spots typical of each particular disease developed on the fully expanded, exposed leaves; more frequently on the laminae and less so on the veins. On the leaves of newly formed shoots, no disease symptoms developed on dolichos bean, haricot bean, cowpea and soybean but on their main stems, which received the bacterial suspension, the usual oval or elongated lesions developed. In lucerne and jute a few newly formed leaves showed vein lesions somewhat similar to those developing on cotton, together with lesions developing at the leaf margins. In castor, vein infection was pronounced and closely resembled that of cotton. The typical angular or roundish spots of the castor disease did not appear on the newly formed leaves but, instead, long brown water-soaked lesions developed on one or

all of the five main veins of the palmate leaf (Pl. 5, figs. 4, 5). The lobes dependent upon infected veins became yellow, outrolled and flaccid. Infection usually spread back to the petioles but no farther. All the leaves produced on the first node of the new shoots died. Higher up the shoots, infection gradually became less severe and in no instance was the main stem of castor infected.

The results of inoculations into cotton were similar to those previously described for bud inoculations.

In further inoculation experiments, dolichos bean inoculated with *X. phaseoli*, castor inoculated with *X. ricini* and cotton inoculated with *X. malvacearum* were used. In dolichos bean the stem was infected but no vein lesions occurred. In castor, typical vein lesions were produced but the stems were not infected. In cotton, stem and vein lesions were produced. The detailed results of inoculations into cotton closely resembled those reported in previous sections.

Pin-stab inoculations were made into the central veins of about thirty mature leaves (leaflets in dolichos bean) and in approximately the same number of young expanding ones.

Infection of leaf veins of dolichos bean appeared in 7 out of 30 mature leaves and 11 out of 26 expanding leaves as small, oval, brown lesions surrounded by pale yellow areas about 1 cm. in diameter extending into the adjacent ground tissue. Infection in castor appeared on 17 out of 31 veins of mature leaves and 16 out of 28 of expanding ones. The lesions were elongated and dark brown. They differed from those developing by bud inoculation in being local, superficial and not accompanied by outrolling of the leaf lobes. In cotton, typical vein lesions were produced on some veins of the inoculated expanding leaves. On the mature leaves, only local narrow lesions developed.

Inoculations were also made into germinating and dormant seeds of the three crops in the same way as previously described for cotton seeds.

In delichos bean, 64 out of 72 seedlings raised from inoculated germinating seed were infected. Infection also appeared on 71 out of 79 seedlings raised from seed inoculated before germination. Infection showed itself on the simple leaves (prophylls) of the plants as narrow, red-brown lesions on the veins, about 0·3–0·6 cm. long surrounded by broad yellow haloes (Pl. 5, fig. 3). The chlorotic areas became flaccid and the whole leaf withered and fell off prematurely. The typical round spots that usually develop in the ground tissue of the leaves were not observed. On the trifoliate leaves no disease symptoms appeared. Oval or elliptical, red-brown, slightly raised lesions, 2–4 mm. in length and surrounded by water-soaked margins developed on the hypocotyls and, to a much less extent, on the first internodes between the cotyledons and the simple leaves. None of the seedlings died as a result of infection.

On castor, 70 out of 78 seedlings from inoculated germinating seed and 42 out of 78 seedlings from seed inoculated before germination were infected. Infection appeared on the cotyledons as round or slightly angular water-soaked spots similar to those normally occurring on the palmate leaves. A few of the infected cotyledons also exhibited vein infection. The hypocotyls of all infected seedlings showed long,

depressed, water-soaked brownish lesions but the stems and leaves above the cotyledons were free of any sign of infection (Pl. 5, fig. 6). None of the plants was killed.

Infection in cotton showed the same trends as in the similar seed inoculation experiment described earlier. A number of heavily infected seedlings died as a result of infection spreading back to the stem and killing the terminal bud.

Discussion

Wickens (1956) and Last (1958) suggested that the bacteria migrate through the vascular elements from stem lesions to the leaf veins or vice versa, on the grounds (i) that new infections appear on the veins under conditions unfavourable to aerial dissemination of the bacteria, (ii) that the vascular tissue of infected plants is discoloured, though the discoloration is not necessarily continuous, and (iii) that the progression of symptoms suggests a vascular disease.

The artificial production of the atypical symptoms of the disease by seed inoculation and by inoculation below stem apices is confirmed by the present study. By bud inoculation, a method which has not been previously attempted, similar atypical symptoms of the disease can be produced on the new shoots. In any of these methods, infection of an actively growing tissue or of a telescoped organ takes place.

In castor, similar unusual symptoms may be produced by *X. ricini*, which causes bacterial leaf blight. In this disease, the stem is not infected even by piercing just below the apex. Vein lesions appear only if germinating seeds or open buds are inoculated.

The production of vein lesions in castor suggests that the pathogen does not necessarily pass through the stem to cause the atypical symptoms. Thus in bud inoculations in cotton, as well as in castor, it would seem that the bacteria reach the leaf primordia and infect the young veins. Even in a later stage, when the young leaf attains its plicate character, the palmately arranged veins are exposed and relatively well developed. The lamina at this stage is actually protected by the veins.

By inoculating the bacteria immediately below the stem apex, *X. malvacearum* reaches the apical bud, infects the bud axis and/or leaf primordia and the subsequent development of the bud into shoot separates infection into portions on the resulting growth. This condition does not take place in castor, whose stem is not naturally infected by *X. ricini*, and the bacteria therefore are not able to reach the apical bud.

In infected seedlings raised from inoculated, germinating seeds, vein lesions develop on the cotyledons together with the usual spots. The veins and mesophyll are equally exposed to infection during emergence from the soil. Infection in castor does not spread from the cotyledons and hypocotyl to the stem but ceases at the cotyledonary node. The true leaves are thus not infected. In cotton, infection reaches the stem apex either from infected cotyledons or hypocotyl, resulting in the appearance of vein lesions on a number of leaves.

Vein lesions in dolichos bean differ in appearance from those of castor and cotton. They are much smaller and do not cause rolling of the leaf. There are indications that they develop in the same way as in castor and cotton, but because of differences

in the structure of the host and the pathogenicity of the causal bacterium, vein lesions in dolichos bean are more restricted in size and distribution. They are therefore inconspicuous and differ very little from the usual characteristic spots of the disease.

The above interpretation of the development of vein lesions by infection of embryonic tissue, in contrast to the vascular movement of the bacteria proposed by previous investigators, is supported by the following:

(i) The development of vein lesions with the associated symptoms depends, essentially, upon infection of actively growing tissue. Inoculation into a fully grown tissue (leaf vein or stem) does not cause this type of infection even when the bacteria are inserted into the vascular tissue by piercing.

(ii) The number of infected leaves developing from an inoculated bud is always limited. Had this condition been caused by the movement of the bacteria in the vascular system, it would be expected to appear in an indefinite number of leaves. The number of infected leaves usually corresponds to the number of embryonic leaves in the bud at the time of infection.

(iii) In cotton, the number of leaves showing vein lesions when the bacteria are inoculated below the stem apex (terminal bud) is considerably smaller than that showing petiole infection. If the axillary buds are inoculated the reverse takes place. These variations in the development of the atypical symptoms may be correlated with the path of entrance into the bud, i.e. the bud axis in the first instance and the veins of leaf primordia in the latter.

(iv) The atypical symptoms develop more readily by inoculating open buds than closed ones, and into germinating seeds than into dormant seeds, indicating that the lesions result from direct contact between the bacteria and exposed tissue at the time of inoculation or shortly after.

(v) The consistent failure to isolate the bacteria beyond visible symptoms suggests that infection is more likely to be local than systemic.

(vi) The failure to demonstrate the bacteria in the vascular tissue beyond the infected area in transverse sections. The angular leaf-spots are normally bounded by the rather thick-walled compact cells of the vascular sheaths of the veins. In vein lesions this tissue is attacked. This is in agreement with the findings of Thiers & Blank (1951) who made an extensive study of the histology of the disease. It may add to the evidence that the veins are infected at an early stage before the vascular sheaths become well developed. Furthermore, inoculations made by piercing into veins of mature leaves are not successful compared with those into veins of young leaves, which readily cause vein lesions.

The statement made by Bryan (1932) that transverse sections of vein lesions show a heavy infection in the vascular system and bacteria pour out in great numbers from the cut ends of the veins is vague, especially as no reference is made to the methods adopted. However, the bacteria have been found in the present and in some previous investigations (e.g. Massey, 1927) to occupy a few wood cells, but only in advanced severe infections of the veins and stem. This is common in necrotic diseases and does not necessarily mean that the bacteria move in the vessels.

The suggested interpretation helps in clarifying the observations of cotton breeders at Shambat that atypical symptoms are more likely to appear after the end of the rainy season on the more resistant types of cotton. Following the rainy season, from the end of September to early November, when the aerial dissemination of the bacteria is more or less checked, new shoots are produced from the axillary buds, some of which have previously been infected during the rains. The new shoots therefore carry a number of leaves showing vein lesions. This condition gradually subsides, and disappears completely towards the end of the growing season. The atypical symptoms are more likely to appear on resistant types because bud infection in this case is usually mild and does not cause the death of many buds as may be the case in the susceptible cottons.

Cultural and cross-inoculation studies have shown that the typical and atypical symptoms of the angular-leaf-spot disease of cotton are caused by one and the same strain of the pathogen. Further, the production of atypical symptoms is not a peculiarity of the cotton disease but may be produced in some other diseases under similar conditions.

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Comment by W. J. Dowson

Dr Sabet's paper on the production of atypical symptoms of infection of the vascular system of cotton and of other plants by inoculation of the buds and young shoots with *Xanthomonas* spp., together with his demonstration that the bacteria do not usually invade the xylem vessels, confirms the work of Thiers & Blank (1951).

By his histological examination of bud inoculations Dr Sabet has shown that the 'atypical' symptoms of vascular spread are due to the attack by the bacteria on young, actively growing tissues of the growing points, i.e. the primordia of leaves and the stem apex, which are chiefly composed of cellulose and in which little differentiation has yet taken place. The subsequent growth of these inoculated primordia leads to the dispersal of the bacteria into separate masses, some of which attack *all* the elements of the *young* leaf while others are confined to the tissue of the *young* bundles of the developing shoots.

Many years ago Smith (1920) observed this vascular infection and noted 'infection takes place very early, i.e. when the leaves are quite small, and then the disease is generally confined to the larger veins or ribs of the leaf'. Subsequent investigators (Bryan, 1932; Wickens, 1956; Last, 1958, 1959) have also called attention to this 'unusual' type of infection. Last (1959) in his experiments on the invasion of *X. malvacearum* when inoculated into the main vein of leaves observed that 'the bacteria spread more rapidly along vascular tissue than into the mesophyll in young leaves than in old and that the initial invasion was quicker in young leaves of young plants than in young leaves of old plants'. One notes that it is the *young* tissues that are most affected.

None of these investigators made any histological examinations of the invaded

tissues but one surmises that they had in mind an invasion chiefly of the xylem vessels, which is unlikely in view of Dr Sabet's work, confirming as it does that of Thiers and Blank.

The term 'vascular infection' needs to be more clearly defined and a distinction should be made between the above described infection in which *all* the elements of the bundles (phloem, xylem and parenchyma) can be attacked and destroyed and infections of the xylem vessels alone, at least in the earlier stages of such disease as are due to *X. campestris*, *Erwinia salicis*, *Pseudomonas solanacearum* or *Corynebacterium sepedonicum*.

A further question arises as to how or by what means does *Xanthomonas malvacearum* destroy the tissues it attacks. The writer has grown some species of *Xanthomonas*, including *X. malvacearum*, in a semi-liquid medium containing salts and filter-paper pulp, which is digested, suggesting that these bacteria secrete cellulases. None, however, will liquefy pectate gel.

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EXPLANATION OF PLATE 5

Fig. 1. Infection on cotton seedlings raised from inoculated germinating seeds; *a*, showing infection reaching the stem from the cotyledons before elongation of first internode (apex killed); *b*, showing infection reaching the stem after the elongation of 1st internode (no vein lesions on the true leaves); *c*, showing infection reaching the stem when the first internode is only a few millimetres long (vein lesions on true leaves); and *d*, healthy seedlings. ($\times \frac{1}{3}$.)

Fig. 2. Infected Domains Sakel leaf from a shoot developing from an inoculated open bud, showing vein lesions (unusual symptoms). ($\times 1\frac{1}{2}$.)

Fig. 3. Simple leaf of dolichos bean from a seedling raised from germinating seed inoculated with *X. phaseoli* (small vein lesions). ($\times 1\frac{1}{2}$.)

Fig. 4. Upper surface of a castor leaf showing vein lesions (unusual symptoms) caused by *X. ricini*. ($\times 1\frac{1}{2}$.)

Fig. 5. Lower surface of a castor leaf with outrolling of lobes, showing vein lesions. ($\times 1\frac{1}{2}$.)

Fig. 6. Castor seedlings showing infection by *X. ricini* on the hypocotyls only. The seedling on the extreme right is healthy. ($\times \frac{1}{3}$.)

(*Received* 8 *December* 1959)

Fig. 1

Fig. 6

Fig. 2

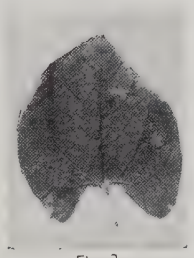

Fig. 3

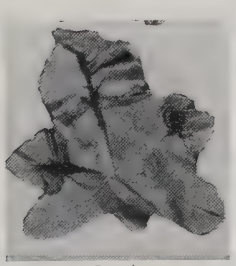

Fig. 4

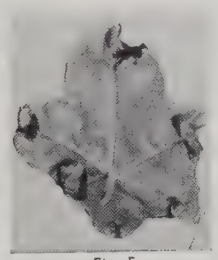

Fig. 5

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INVESTIGATION OF FIRE-BLIGHT OF PEAR IN ENGLAND

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(With Plate 6 and 3 Text-figures)

Outbreaks of fire-blight, caused by *Erwinia amylovora*, in Kentish orchards during 1957 constitute the first authenticated record of the disease in Europe. The origin of the disease is unknown, but evidence suggests that it was not present in the orchards prior to 1957.

The seasonal development and symptoms of the disease are described in detail. Fire-blight attacked trees only during the growing season; infection began in the blossoms, particularly summer blossoms, and spread into the branches forming cankers, in many of which the pathogen remained viable during the winter 1957–8. In the orchards examined there was a higher incidence of new infections by July 1958 on trees attacked the previous year than on those which were then healthy, and this is discussed in relation to overwintering sources of inoculum. The distribution of the disease in orchards during 1957 and 1958 was consistent with spread by pollinating insects rather than rain.

The outstanding susceptibility of the variety Laxton's Superb in all localities was attributed partly to its tendency to produce abundant secondary blossom. Inoculations of blossoms, fruits, shoots and branches showed it also to have less inherent resistance than the varieties Williams's Bon Chrétien and Conference, neither of which has been seriously affected in the field. The progress of symptoms following inoculation of pear and infection experiments with apple, *Cotoneaster* sp., *Pyracantha* sp., and other hosts, are described.

A comparison of climatic conditions favouring the disease in the U.S.A., i.e. high temperatures and high humidity, with meteorological records at East Malling, suggests that epidemic infection of spring blossom would be exceptional in this country.

Introduction

In the autumn of 1957, trees of the variety Laxton's Superb in pear orchards of west Kent were found to be dying from extensive cankers present on the stems and branches. During 1958 it was proved that they were suffering from fire-blight disease caused by the bacterial pathogen, *Erwinia amylovora* (Burrill) Winslow *et al.* (Crosse, Bennett & Garrett, 1958).

Fire-blight, the most destructive of apple and pear diseases, is a native of North America where it has been known for over 150 years. It probably occurred there on wild rosaceous hosts before the introduction of cultivated apple and pear varieties from Europe. It was first reported from the Hudson River highlands in 1780 and was later discussed as an important feature of pear cultivation by William Coxe in his book *View of the cultivation of fruit trees and the management of orchards and cider*, published in the U.S.A. in 1817. The term 'fire-blight' was already in use at this time and Coxe mentions specifically the sudden browning of the leaves

of diseased trees 'as if passed through a hot flame'. In the years preceding and following Burrill's (1881) proof of the bacterial nature of the disease, it spread steadily westwards, reaching California by the turn of the century. It was reported at about the same time from Canada where it is now fairly widely distributed.

Fire-blight was identified in New Zealand by Waters in 1922 and until the present outbreak in Britain this was the only authenticated record of the disease outside North America. It first appeared in 1919 in several widely scattered areas in the Auckland province and in the next 7 years, despite all efforts to prevent it, spread gradually southwards, reaching the Kaikoura and Christchurch districts of the South Island by 1929. The history of fire-blight in New Zealand has been discussed by Reid (1930), Cunningham (1925) and by Curtis (1934).

Fire-blight may occur in Asia; it has been reported from Japan as early as 1911, and more recently has been stated to be present in China (Ciferri, 1955) and Jordan (Vestal, 1954).

Workers in Europe have long been aware of the dangers of fire-blight and by 1932, when the position was reviewed by Pethybridge (1934), it had been reported from Germany, Switzerland, Italy, France and Sicily. More recently the disease has been said to occur in Roumania (Veresciaghin, 1940) and Poland (Rojecka, 1958). In some of these cases bacteria were isolated from infected tissues, but as no proof of identity or pathogenicity was offered, there may have been confusion with *Pseudomonas syringae*. This organism, under various synonyms (see Elliott, 1951) has been described as a cause of blossom blight of pear in several countries including Britain (Wormald, 1946), U.S.A. (Clara, 1932; Wilson, 1934), Canada (McKeen, 1955), South Africa (Doidge, 1917) and France (Ridé & Sutić, 1958). *P. syringae* is usually confined to the blossom, but it may kill the fruiting spurs and spread into the branches causing cankers; Wilson (1936) has described a winter canker phase of this disease on the larger branches and trunks of pear trees. Nearly all accounts of blossom blight due to *P. syringae* refer directly or indirectly to the possibility of confusion with fire-blight.

Symptoms of fire-blight in Britain

In all essential features the disease in Britain has agreed closely with descriptions from the U.S.A. and New Zealand. It attacks trees only when they are actively growing and infection usually begins in the blossoms; it then progresses rapidly into the branches, forming cankers which may reach the trunk of the tree by the end of the season. Cankers remain dormant during the winter, but in some of them bacteria survive until the following spring when further extension of the canker may take place. In the U.S.A. these overwintering, or 'holdover', cankers are regarded as the main source of inoculum for the first (primary) infections of blossom the following year. Accounts of fire-blight in the U.S.A. are given by Thomas & Parker (1933), Thomas & Ark (1934*a*) and Anderson (1956). Symptoms in England have been illustrated in a brief popular account of the disease by Crosse, Bennett & Garrett (1959). The following detailed descriptions are now offered.

The flower stalks of recently infected blossoms are light brown and may appear varnished due to the spread of bacterial exudate over the surface. Eventually the whole flower truss shrivels and turns black. In the meantime the infection progresses down spurs or twigs into the larger branches and spreads rapidly in both directions. Leaves in the affected area lose their shine and develop marginal scorching. The presence of small groups of such leaves on trees is usually one of the first obvious signs of fire-blight in the field. Gradually the leaves turn completely black, and as more are involved, the branches take on the typical scorched appearance which gave the disease its name. The diseased branches now stand out starkly against a background of green foliage but in the more serious cases the whole orchard may appear as though swept by fire.

The progress of the canker in the branch is marked by a dark grey discoloration of the bark, contrasting sharply with the normal brown of a healthy branch. If a tangential cut is made in a diseased area the deeper seated tissues are still white, but overlaid by a thin, wet-looking, brown layer immediately under the surface of the periderm. This appearance is characteristic and indicates infection mainly in the cortical tissues. The discoloration of the cortex is less intense in the younger parts of the lesion and the advancing edge of the canker may merge almost imperceptibly into the green of the healthy tissues.

Towards the end of the growing season progress of the cankers is checked, although by this time they may have entered the trunk of the tree. The margin of the canker now becomes clearly defined as an undulating line separating the slightly depressed, dark grey diseased areas from the healthy bark. Some cankers may retain this appearance throughout the winter, but in others deep cracks are formed along the margins which often extend, tongue-like, into the healthy area of the branch. Longitudinal or slightly oblique cracks sometimes appear on the main body of the canker giving rise in extreme cases to an intersecting network of fissures in the bark. On dormant cankers on the larger branches there is a tendency for the bark to wrinkle and peel off from the underlying dead tissues in thin, pinkish scales. This sometimes extends to discrete areas of yellow moribund tissue, isolated during the spread of the canker along the branch. Most of the cankers extend for several feet; some however, for example those resulting from late summer infection of secondary blossom, may be sealed off soon after entering the branch, leaving a small elliptical canker round the point of entry. Small cankers were also a feature of an isolated outbreak on Laxton's pear in the West Midlands, but this is believed to have been related to the weak state of tree growth rather than to time of infection.

Overwintered cankers present much the same appearance in spring as in the preceding autumn, except for the occurrence of fructifications of secondary fungal invaders, common amongst which was *Pezicula corticola*, reputed to cause a surface bark canker in pear. The tissues in the diseased areas are stained deep brown as far as the wood and frequently smell strongly of fermentation. Small, brown streaks, extending beyond the original limit of the canker, are the first signs of renewed activity in the spring. These eventually develop into a continuous line of

infection advancing into the adjacent healthy tissues. At first the newly invaded areas are dark brown and often show concentric zoning, suggesting that some factor is operating, possibly low temperature, as a frequent check to the invasion in its initial stages. This effect soon disappears and the spread of the infection becomes more uniform. This again tends to be confined to the surface layers of the bark, but unlike newly established summer cankers, the tissues become 'foxy-red', merging through a diffuse mottled red-green area into the healthy bark. Several trees which survived the attacks in 1957 with the loss of only one branch were killed early in 1958 by the extension of holdover cankers present in the trunk. These trees were conspicuous by their yellow foliage or by the shrivelling of the leaves before they were fully expanded (Plate 6, fig. 1).

Infection of shoots was common in all diseased orchards during 1958. The first signs are a light-brown, oily discoloration of the surface tissues of the shoot axis and a slight chlorosis of the leaves. The axis turns black and there may be invasion of the petioles and midribs of the leaves. The laminae are not usually invaded, except, characteristically, for a narrow wedge of tissue at the point of attachment with the petiole. Eventually the leaves turn brown and the whole shoot withers.

Infected fruits are first oily brown, but soon wrinkle and turn black like dried prunes; in humid weather they exude an ooze which spreads on the fruits, giving the surface the oily appearance. When mature fruits are infected, later in the season, circular brown lesions are formed confined to one side of the fruit.

Direct infection of shoots, named 'twig blight' by American authors, was occasionally observed giving rise to cankers in the branches. Most shoots and fruits, however, became infected by spread from cankers in the branches.

History of outbreaks in Britain (1957–8)

The disease first came to notice in September 1957 on the variety Laxton's in a 12-acre orchard at Loddington, about 2 miles south of Maidstone in Kent. In the next few weeks similar symptoms on the same variety were reported from four other farms in Kent, two south of Maidstone, at Loose and East Farleigh, one at Ash near Wrotham, and the other at Higham near Rochester. A survey by the National Agricultural Advisory Service revealed nine further outbreaks in Kent during 1958, including one near Swanley where the disease had obviously been present in 1957. As a result of this survey it now appears that there were three main foci of the disease in south-east England by the end of 1957, centred near Maidstone, Rochester and Swanley (Lelliott, 1959).

Although die-back of branches first attracted attention to the disease in the autumn of 1957, it was subsequently established that infected summer blossoms had been seen in July of that year in some of the affected orchards. No special significance was attached to this at the time, but it now seems clear that the widespread distribution of the disease, as witnessed later in the season, was then taking place.

Renewal of canker activity in the spring of 1958 approximately coincided

with bloom on the variety Laxton's and was first observed on 28 April as small necrotic streaks extending into the healthy tissues for a distance of 1–3 cm. from the margin of the canker. By the beginning of June the cankers had spread considerably and the typical foxy-red discoloration of the surface layers of the bark could frequently be detected more than 12 in. beyond the overwintered margin of the canker. The trunks of many trees had now been girdled and were showing yellowed foliage and withered branches.

Diseased flower trusses, mostly summer blossoms formed after the main period of spring flowering, were first seen on 26 June, but infection had obviously taken place earlier as cankers up to 40 cm. in length were already present on some of the branches. Infection of summer blossoms continued throughout the summer of 1958 on the variety Laxton's with the result that the disease spread progressively in many of the affected orchards. This feature will be described later in relation to the distribution of the disease in the field.

Infection of primary or spring blossom, at the end of April or beginning of May, was observed on Laxton's and Conference in one locality, but was comparatively rare, especially on Conference; epidemic spring infection of the kind described from the U.S.A. was not observed during 1957 and 1958 in this country.

Host range of *Erwinia amylovora*

By the end of 1958 fire-blight had been found only on pear and on quince rootstock suckers in diseased orchards. During 1959, however, isolated cases were confirmed by officers of the N.A.A.S. and Plant Pathology Laboratory, Harpenden, on hawthorn, *Cotoneaster* spp. and *Sorbus* spp. So far it has not been reported on apple.

The host range of *Erwinia amylovora* has been extensively investigated in North America (Snow, 1922; Rosen & Groves, 1928; Pierstorff, 1931; Thomas & Thomas, 1931). Smith (1931) successfully infected various *Juglans* spp. by inoculation, but in nature fire-blight is restricted to plants of the Rosaceae. According to Thomas & Ark (1934*a*) there are more than 120 susceptible species in this family, including representatives from each of the subfamilies Spiraeoideae, Rosoideae, Prunoideae and Pomoideae. Fire-blight, however, is primarily a disease of the Pomoideae and overwintering of the pathogen on plants outside this subfamily is apparently unknown (Thomas & Ark, 1934*a*). In addition to the economically important *Pyrus* (pear) and *Malus* (apple) the following are among the susceptible genera of the Pomoideae: *Amelanchier* (service berry), *Chaenomeles* (flowering quince), *Cotoneaster*, *Pyracantha* (firethorn), *Eriobotrya* (loquat), *Crataegus* (hawthorn), *Mespilus* (medlar), *Cydonia* (quince), and *Sorbus* (mountain ash). Plants of these genera may act as sources of infection for fruit crops, but apparently not all are equally important in this respect. In New York State, for example, the disease is seldom seen outside fruit orchards except on hawthorn, firethorn and mountain ash (Thomas & Parker, 1933; Parker, Fisher & Mills, 1956). In New Zealand the natural hosts of fire-blight are apple, pear, quince, medlar, loquat, firethorn and hawthorn (Curtis,

1934). Pear and hawthorn are described as especially susceptible, and the presence of untrimmed hawthorn hedges bearing blossom is considered to have been an important factor in the spread of fire-blight throughout the Dominion. The practice of trimming hawthorn hedges may reduce the importance of this host in this country, but until there is more evidence on this point hawthorn must be regarded as a special hazard in attempts to control or eradicate the disease in England.

Susceptibility of pear and apple varieties

The incidence of fire-blight on pear varieties in an orchard at East Farleigh at the end of 1957, and in orchards at Loddington, Ash and Wainscott at the end of 1958, is shown in Table 1. The pattern of varietal susceptibility illustrated by these data has been repeated with minor variations in all affected orchards.

Table 1. *Incidence of fire-blight on different pear varieties in four commercial pear orchards at the end of* 1957 *or* 1958

	E. Farleigh		Loddington		Ash		Wainscott	
Approximate acreage ...	9		12		7		1·3	
Age of trees (years) ...	8		9		7		8	
Variety	Total trees	% infected	Total trees	% infected	Total trees	% infected	Total trees	% infected
Laxton's Superb	426	81·5	666	40·5	289	32·2	112	28·6
Conference	1031	0·4	1527	1·1	711	0	—	—
Williams's Bon Chrétien	162	0	7	0	294	0	—	—
Doyenné du Comice	—	—	—	—	—	—	208	0·5
Packham's Triumph	—	—	—	—	112	0	—	—
Bristol Cross	418	1·9	—	—	—	—	—	—

The outstanding feature of the fire-blight outbreaks in England has been the concentration of the disease on the variety Laxton's. At East Farleigh, for example, more than 80% of the Laxton's trees had been attacked by the autumn of 1957, the incidence on Conference at this time being less than 1%, while Williams's Bon Chrétien was completely free of the disease. A few infected trees of the latter variety were found in other orchards, including a second orchard at Loddington consisting of about half an acre of mature trees of Laxton's with a few Williams's trees interplanted. This orchard was virtually destroyed by the disease during 1958. Williams's was also affected in another orchard near Rochester but not very severely. In the same locality a severe attack on Fondante de Thiriott occurred in the summer of 1958, nearly fifty trees, representing about half the total planting of this variety, being badly damaged.

Reasons for the extremely high incidence of the disease on Laxton's compared with other varieties will be discussed later. Laxton's is not grown commercially in the U.S.A. and New Zealand and the only previous evidence of its susceptibility derives from a pear variety trial in Ohio, U.S.A., where it was ranked with Williams's and other varieties as very susceptible (Howlett, 1957). Williams's (syn. Bartlett) is widely grown in the U.S.A. and is regarded there as amongst the most susceptible varieties, a group which also includes Flemish Beauty, Beurré Bosc, Doyenné

du Comice and Packham's Triumph. The latter two were also present in affected English orchards, but like Williams's, Comice was not seriously affected and Packham's Triumph not at all (Table 1). In New Zealand Williams's, Packham's Triumph, Vicar of Winkfield, Louise Bonne Jersey, Comice, Clapp's Favourite, Josephine de Malines, Beurré Bosc, Glou Morceau and Marie Louise are considered to be particularly susceptible (Hamilton, 1959).

Although pear varieties vary in susceptibility, none of high quality is markedly resistant. This reflects the fact that most of them are derived from *Pyrus communis*, a European species found by Reimer (1925, 1950) to possess few natural sources of resistance.

Fire-blight is a less serious problem on apple than pear. Differences in varietal susceptibility in the U.S.A. (Thomas & Parker, 1933; Miller, 1929) are not of major interest in England, because, unlike pear, there are few commercial apple varieties common to both countries. The only exception of note, Jonathan, has been described as 'moderately susceptible' (Brooks, 1926) to 'highly susceptible in certain States' (Parker, Fisher & Mills, 1956). According to Hamilton (1959), Jonathan is also susceptible in New Zealand, but the only English variety mentioned, Cox's Orange Pippin, is said to be little affected.

Development and spread of fire-blight in orchards (1957-8)

Data in Table 2 shows for the orchards of East Farleigh, Loddington and Ash, the numbers of Laxton's trees (1) infected during 1957, (2) infected 1957, re-infected through blossom 1958, (3) infected in 1957, with holdover cankers becoming active in 1958, (4) infected for the first time during 1958. The orchard at Loddington was considered in two parts, viz. (*a*) the *main* orchard of approximately 11 acres, with the Laxton's trees in rows running east and west and alternating with three successive rows of Conference, and (*b*) a *subsidiary* block of about 1 acre consisting entirely of Laxton's, continuous with the western periphery of the main orchard (see Text-fig. 1). Details of acreage and varieties in the other two orchards are given in Table 1.

In 1957 the disease was most serious in the orchards at East Farleigh, over 80% of the trees being affected. It was also serious in the subsidiary block of Laxton's trees at Loddington, but had only just begun to make inroads into the main orchard by the end of 1957. At Ash slightly over 30% of the Laxton's were infected during 1957, but because the trees were younger, the mortality was proportionately very much higher than in the other orchards.

In spring and early summer of 1958, many of the trees infected the previous year had holdover cankers on stems and branches, which were extending rapidly and killing the trees. The exceptions were the subsidiary block at Loddington, where tree surgery had been extensively practised during the winter 1957–8, and Ash, where many of the trees had been killed in 1957.

New infections, mainly of spring and early summer blossoms, were present in most orchards by July 1958 on trees which had survived infection in 1957. By

this time also many new trees had become infected, particularly in the main orchard at Loddington. The most significant development here, however, was an increase in newly infected trees, from 30 to 109, during the period July to September, as a result of infections of summer blossoms. At Ash there was little new infection in 1958. This was attributed to one or all of the following factors: (*a*) few sources of inoculum in the orchard due to the high mortality of trees in 1957, all of these were removed in the winter 1957–8; (*b*) sparse development of blossom during 1958; and (*c*) grassing down of the orchard in the winter 1957–8. Clean cultivation and any other factors stimulating vigorous tree growth are well known in the U.S.A. to increase susceptibility.

Text-fig. 1. Distribution of fire-blight in a pear orchard at Loddington, Kent, during 1957 and 1958. •, Trees infected 1957; ✧, trees infected by July 1958; ✲, trees infected by September 1958; L = Laxton's Superb rows. For further description see text.

Relationship between infections in 1957 *and* 1958

American authors have repeatedly stressed the importance of overwintering 'holdover' cankers as sources of initial or primary blossom infections in the spring. Tullis (1929), for example, illustrated the distribution of early blossom infections in an apple orchard round a single holdover canker in the orchard. Parker (1936)

was able to demonstrate in a pear orchard a tendency for blossom infections to be concentrated in trees carrying holdover sources. No records are available of primary infections of blossoms in English orchards in spring 1958. Records of infections made in July, mostly of spring and early summer blossoms, were therefore compared with infections the previous year (Table 3).

TABLE 2. *Distribution of fire-blight infections on Laxton's Superb pear trees in Kentish orchards during* 1957 *and* 1958

		Trees infected 1957			Trees newly infected 1958		
Orchard	Total no. trees	Total	Re-infected 1958	Holdover cankers active 1958	By July	By Sept.	Total
Loddington							
(*a*) Main orchard	540	68	12	13	30	79	109
(*b*) Subsidiary block	126	78	21	0	5	10	15
East Farleigh							
(*a*) Orchard 1	301	242	34	49	3	—*	3
(*b*) Orchard 2	125	100	6	30	1	—*	1
Ash	289	92	1	7	0	1	1

* No record.

TABLE 3. *Relationship between new infections in July* 1958 *and trees of Laxton's Superb infected in* 1957 *at Loddington and East Farleigh*

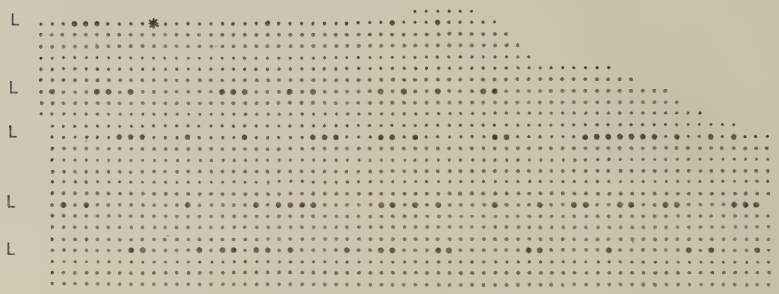
Orchard	Percentage trees infected 1957, re-infected 1958 (A)	Percentage trees healthy 1957, infected 1958 (B)	Ratio A/B
Loddington			
(*a*) Main orchard	17·7	6·4	2·8
(*b*) Subsidiary block	30·0	10·4	2·9
East Farleigh			
(*a*) Orchard 1	14·0	5·1	2·8
(*b*) Orchard 2	6·0	4·0	1·5

It was clear that a higher proportion of the infections recorded by July 1958 were on trees that had been infected the previous year. As there was nothing to suggest that these trees were innately more susceptible, this distribution probably reflects the presence of holdover sources in the trees infected during 1957. If this is true then it suggests that there were overwintered sources of infection additional to active holdover cankers, for there were none of these in the subsidiary block at Loddington (Table 2) and little obvious relationship between the numbers present in other orchards and the distribution of new infections. Possibly many of the cankers in the orchard, which did not become active again in 1958, may have carried overwintered inoculum.

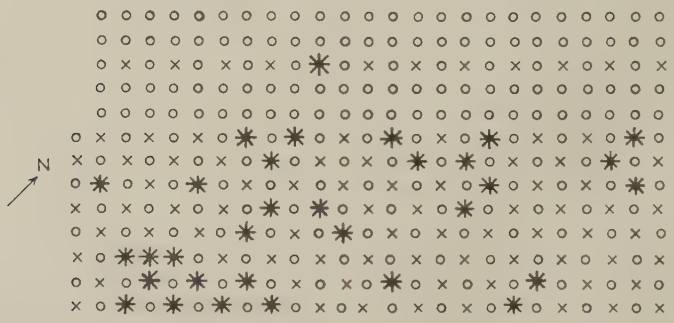
Spread of fire-blight in individual orchards (1957–8)

The distribution of diseased trees in three orchards (1) at the end of 1957, (2) by July 1958, and (3) by September 1958, are shown in Text-fig. 1–3.

At Loddington (Text-fig. 1) the disease was concentrated in the western half of the orchard in 1957 and probably originated in the subsidiary block of Laxton's. By September of the following year it was widely and more or less uniformly distributed in the main orchard almost entirely on the Laxton's, although a few Conference trees were infected here and there. At Ash (Text-fig. 2), for reasons



Text-fig. 2. Distribution of fire-blight in a pear orchard at Ash, Kent, during 1957 and 1958. •, Trees infected 1957; ⁎, tree infected between July and September 1958; L = Laxton's Superb rows.



Text-fig. 3. Distribution of fire-blight in a pear orchard at Wainscott, Kent, during 1958. ⁎ Infected trees; ○, Doyenné du Comice; ×, Laxton's Superb.

already suggested, there was virtually no development in 1958, but the distribution in 1957 again was fairly uniform. Wainscott (Text-fig. 3) was of special interest because the disease first entered this orchard (Table 1) during 1958, probably from a neighbouring orchard lying about $1\frac{1}{2}$ miles to the north-west where the disease had been present in 1957. It apparently started in the southern corner of the

orchard, for the symptoms here were the most advanced when the outbreak was first discovered. From this focus it invaded every part of the orchard, with an apparent tendency for infected trees to occur in groups.

The most notable feature of the over-all distributions in these orchards was the absence of any obvious disease gradients. Hence rain cannot have played a major part in the widespread dissemination of inoculum. On the other hand, the pattern of development appears consistent with spread by pollinating insects. The importance of the latter, particularly honeybees, in spreading bacteria from blossom to blossom (secondary infection) was first demonstrated by Waite (1902), and has since been confirmed by many workers. Gossard & Walton (1922) and Miller (1929) amongst others, obtained evidence that local spread of secondary infections may occur in rain, but the present view appears to be that expressed by Parker (1936), namely that rain is important in the establishment of blossom infections from hold-over cankers (primary infection), but is less important than pollinating insects in the spread from blossom to blossom.

Experimental

A full description of the English isolates of *Erwinia amylovora*, including a study of bacteriophage reactions, is in course of preparation. Methods for the isolation of the organism and characters most useful for laboratory identification are described by Billing, Crosse & Garrett (1960).

The following is an account of inoculation experiments using detached plant material kept under humid conditions in the laboratory, potted greenhouse trees, and in one instance a healthy tree in an infected commercial orchard. These experiments have confirmed the cross-infectivity of isolates from different host organs.

Blossoms were infected *without* wounding by placing inoculum into the receptacle cup and fruits, shoots and branches by wounding. Shoots and blossoms on potted trees were usually enclosed in polythene after inoculation to maintain humidity. Bacterial inoculum was prepared by suspending growth from a 24 hr. culture on nutrient agar + 2% glycerine slopes into 5 ml. of sterile distilled water.

Pear inoculations

Using Laxton's, Williams's and Conference as experimental varieties all the field symptoms of the disease were successfully reproduced by inoculation, including 'holdover' cankers. These were observed on two greenhouse trees of Williams's and one of Conference. The trees had been inoculated in mid-summer and the resulting cankers were 35, 18, and 5 cm. in length respectively by the time they became dormant in September. The cankers became active again in the spring of 1959, and by May all three trees were dead, the size of the cankers now exceeding 60 cm.

Inoculations of shoots and fruits are of interest in connexion with diagnosis of the disease. In the laboratory, lesions on fruits appear after about 48 hr. as water-soaked areas about 1 cm. in diameter. This extends and is followed by blackening

of the surface tissues of the fruit from the centre outwards. Copious bacterial ooze, at first pearl-coloured but later turning amber, is formed under humid conditions after 2–4 days, particularly at the site of inoculation (Pl. 6, fig. 2). Fruitlets about 2 cm. at their maximum width may be completely rotted in 5–6 days, but with more mature fruits the infection tends to be arrested after a moderate-sized lesion is formed. On cut shoots and shoots on greenhouse trees, the first signs are usually seen after 3–4 days as discrete water-soaked streaks separated from the point of inoculation by regions of apparently healthy tissues. Bacterial ooze is produced after about 5 days, by which time the visibly infected areas are beginning to coalesce and the axis of the shoot to appear uniformly brown (Pl. 6, fig. 3). Later the shoots turn black and there may be invasion of the petioles and midribs of the leaves. Shoot inoculations are less consistently successful than fruit inoculations, even with succulent, actively growing shoots, e.g. water shoots, which are most satisfactory for the purpose.

Development of blossom symptoms

The development of symptoms on blossoms, under humid and warm conditions in the greenhouse with midday temperatures frequently exceeding 70° F., was as follows:

3–4 days: bacterial ooze appearing in receptacle cup around base of stamens.
5–6 days: outer surface of receptacle cup now dark green and waterlogged; stamens withering.
7–8 days: pedicel tissues waterlogged; bacterial ooze present on outer surface of receptacle and petioles (Pl. 6, fig. 4).
9–10 days: diseased tissues turning black and ooze amber in colour.
12–14 days: flower trusses completely blackened; invasion of fruiting spurs and branches beginning.

After 28 days the infection had spread into branches on some trees of Laxton's resulting in cankers up to 22 cm. in length.

Rate of spread of branch lesions

By the time blossom infections were detected in the field, the disease had frequently progressed considerable distances into branches, and trees found to be healthy at one inspection might be showing severe symptoms when examined a few weeks later. This rapid development of cankers under field conditions is illustrated by data (Table 4) from the inoculated orchard tree of Laxton's mentioned above. For both isolates the average rate of advance during the first 19 days was 1·4 cm./day. After a further 22 days the cankers had reached the tips of the branches, a distance in each case of approximately 100 cm. from the point of inoculation. During the second period, therefore, the margins of these experimental cankers were advancing at an average of over 3 cm./day. Six weeks after inoculation all the leaves on the infected branches were withered and brown, giving the tree the scorched appearance characteristic of the disease. At this stage it was indistinguishable from nearby trees with natural infection (Pl. 6, fig. 5).

Comparison of the resistance of the pear varieties Laxton's Superb, Williams's Bon Chrétien and Conference

The susceptibility to infection and the inherent resistance of tissues to spread of infection of these varieties were compared on detached plant material and greenhouse trees. The bacterial isolates were P 56 (ex-Conference), P 7 (ex-Laxton's) and L 56, W 56 and C 56. The latter were re-isolates of P 56 after two passages

TABLE 4. *Maximum distance of spread of cankers (cm.) from point of infection on branches of a field tree of Laxton's Superb, 19 days after inoculation*

Bacterial isolate*	Branch replicate 1	2	3	Mean	Rate of advance (cm./day)
P 32	24	19	48	30·3	1·6
P 50	13	28	29	23·3	1·2
Control	0	0	0	0	0

* P 32 originally from an active branch canker and P 50 from an infected blossom of Laxton's Superb.

TABLE 5. *Results of inoculations to shoots and stems of Laxton's Superb, Williams's Bon Chrétien and Conference pears, with five strains of* Erwinia amylovora

Host material	Bacterial strain	Variety: Laxton's No. infected	Laxton's Av. lesion length (cm.)	Williams's No. infected	Williams's Av. lesion length (cm.)	Conference No. infected	Conference Av. lesion length (cm.)
Shoots	*P 7	7/8	18·9	0/9	0	0/9	0
	*P 56	4/4	22·0	6/6	3·5	3/4	3·0
	†L 56	5/5	14·0	2/5	1·2	5/5	7·9
	†W 56	5/5	15·4	4/5	1·8	5/5	9·6
	†C 56	5/5	14·2	2/5	0·6	5/5	9·6
% infected		96·5	—	46·5	—	64·5	—
Av. lesion length (cm.)		—	16·9	—	1·8	—	7·5
Stems	‡P 7	8/9	4·2	8/9	0·7	3/9	0·2

* On shoots of 3-year-old greenhouse trees after 12 days.
† On cut shoots in the laboratory after 7 days.
‡ Results after 28 days.

through tissues of Laxton's, Williams's and Conference respectively. Results of shoot and stem inoculations are summarized in Table 5. With shoot inoculations the susceptibility of the varieties was in approximately the same relationship as their susceptibility in the field. The comparison between Williams's and Conference, however, was heavily weighted by the results with strains L 56, W 56, and C 56. Laxton's was obviously the least resistant variety, both to infection and to the spread of the disease after infection. Results from stem inoculations were similar. In experiments on fruitlets in the laboratory Laxton's was also the least resistant,

the average lesion size (measured on a 0–5 rating according to size) being 4·4, compared with 3·6 and 3·0 on Williams's and Conference respectively.

Results from blossom infections of Laxton's and Conference are summarized in Table 6. All the inoculated Laxton's blossoms were infected after 10 days and all but 2% of the Conference blossoms after 14 days, but within this period the disease developed more rapidly on Laxton's. There was also 34·4% infection on the uninoculated blossoms of Conference. It occurred on two trees only, and as the experiments were conducted in an insect-proof compartment of the glasshouse, it was attributed to contamination from local showers of pollen, released when these trees were moved 2 days after inoculation.

Table 6. *Percentage diseased blossoms on Laxton's Superb and Conference pear trees at various times after inoculation with* Erwinia amylovora

	Laxton's		Conference	
Days after inoculation:	Inoculated (57)*	Uninoculated control (42)	Inoculated (181)	Uninoculated control (135)
6	49·2	0	32·2	0
10	100·0	0	69·2	1·5
14	100·0	0	98·0	34·4

* Figures in parentheses refer to the total number of blossoms used in the experiments.

Inoculation experiments on alternative hosts

Apple

Blossoms on potted glasshouse trees of Cox's Orange Pippin and the four American varieties, McIntosh, Spartan, Red Delicious and Winesap, were successfully infected with a strain of *E. amylovora* from Laxton's pear. The symptoms were similar to those on pear, except that the flower trusses turned orange-brown instead of black. McIntosh was the least resistant and was the only variety where conspicuous bacterial ooze was formed or where the infection penetrated into the fruiting spurs, although this never exceeded 2 cm. Cox's appeared fairly resistant, a fact which seemed to correspond to its orchard behaviour in New Zealand (Hamilton, 1959).

Shoots of potted trees of Winesap and detached Cox's shoots have also been infected, but shoot infections of apple have been less consistently successful than pear. Inoculations to apple stems and branches all failed, except on McIntosh where a lesion 2–3 cm. was formed after about 4 weeks.

Ornamental trees and shrubs

Plants successfully inoculated with the pathogen were: hawthorn (*Crataegus oxyacantha*), mountain ash (*Sorbus aucuparia* var. *asplenifolia*), quince (*Cydonia oblonga*), Japanese quince (*Chaenomeles lagenaria*), *Pyracantha lalandii*, *Cotoneaster salicifolia*, and *Amelanchier* sp.

The symptoms produced on shoots of these hosts resembled those on apple

in producing a browning rather than a blackening of infected tissues, except hawthorn, where shoots did turn black. Quince and the *Cotoneaster* gave the best results, with lesions averaging 19 and 14 cm. respectively and copious bacterial ooze.

Discussion

Fire-blight is favoured by warm humid weather during blossom and is most serious in those parts of the U.S.A. where these conditions apply. Brooks (1926) described the weather conditions favouring epidemic fire-blight on apple as 'temperatures from 65–85° F., high humidities, cloudy weather, and frequent showers'. Parker, Fisher & Mills (1956) reported similarly on the basis of 35 years' observation. They found that temperature relationships only held if there was at least a trace of rainfall, and they attributed this to the humidity factor. Thomas & Ark (1934*b*) related fire-blight infection of apple and pear blossoms to low concentrations of sugars in the nectar and suggested that high humidity favoured infection by reducing nectar concentration. Ivanoff & Keitt (1941) confirmed and extended these observations, although experiments with bees indicated that other factors in addition to nectar concentration were sometimes limiting (Keitt & Ivanoff, 1941).

The average flowering dates at East Malling Research Station for 10 years, 1950–9, were: Conference, 18 April–3 May and Comice 22 April–12 May. Williams's usually coincides approximately with Conference, and Laxton's with Comice. Apples are about 10 days later than pears, depending on variety and season, but there is considerable overlapping. The average of the means of the daily maximum temperatures at East Malling for the 20 years 1939–58 were: March 50·1° F., April 56·5° F., May 62·4° F., June 68·1° F. and July 71·1° F. In the spring, temperatures higher than the means are rarely accompanied by very high humidity. Assuming that American observations are applicable to this country, then it seems that our climatic conditions will favour infection of summer blossoms but are unlikely to lead to epidemic infection in the spring except perhaps in the extremely susceptible variety Laxton's.

The pattern of varietal susceptibility observed so far in English outbreaks is important because it suggests that the disease would rarely be a serious problem in pear orchards except on the variety Laxton's. One reason for the outstanding susceptibility of this variety is undoubtedly its tendency to produce abundant summer blossoms, but as most pear varieties produce summer blossoms at some time during the growing season, this alone will not account for the extreme differences in varietal susceptibility observed. Neither will it explain the lower incidence of spring blossom infections on varieties like Williams's and Conference, compared with Laxton's.

In inoculation experiments, shoots and branches of Laxton's were more rapidly invaded and showed less resistance to infection than those of Williams's and Conference. Blossom infections also developed more quickly on Laxton's than Conference, although with the high inoculum concentrations employed the final level of infection was approximately the same on both varieties. Hildebrand

(1937) was able to infect nectaries of apple with a single bacterium. Ivanoff & Keitt (1941) concluded that ten bacteria constituted a highly efficient inoculum for pear when introduced into nectar under favourable conditions, but observed an increase in infection using 100 or 1000 cells. This suggests an effect of inoculum concentration comparable to that observed in bacterial canker of cherry (*P. morsprunorum*), where threshold inoculum concentrations were related to the inherent resistance of the variety (Crosse, 1957). If we postulate a similar relationship in pear, then it suggests that the threshold inoculum for Laxton's under any given set of environmental conditions will be lower and the chances of infection higher than for the other varieties. If this argument is extended, it is possible to imagine a situation where environmental factors are so limiting that Laxton's become infected but other varieties do not.

The origin of fire-blight in Britain is still shrouded in mystery. With so susceptible a variety as Laxton's Superb present in pear orchards it seems unlikely that it could have occurred prior to 1957 without attracting attention. Local evidence and orchard observations support this view. Spring blossom was exceptionally early in 1957, beginning at the end of March, and the highest temperature recorded during this period at East Malling, on one day *only*, was 67° F. Most growers saw wilted blooms for the first time in July following an abnormally heavy crop of summer blossoms on Laxton's. From the rate of symptom development observed in inoculation experiments this agreed with the fact that severe symptoms were not witnessed until late August or early September. It seems highly probable therefore that the disease first entered orchards in the summer of 1957. Beyond this is mainly speculation at present. There is little to indicate whether the infection reached one orchard first and then spread rapidly to others, or whether they were all infected independently from the same outside source or sources, or how the disease was transmitted from one focus to another, the distance in one instance exceeding 10 miles. Despite the substantial acreage of pears in east Kent, in which Laxton's is widely planted, fire-blight has not yet appeared in this region. All new cases found in 1959, including many on ornamental hosts in the London suburbs, have occurred west of Maidstone. It could be interpreted from this that the disease originated in London and spread eastwards into commercial pear orchards.

We offer our thanks to the many officers of the National Agricultural Advisory Service and Plant Pathology Laboratory, Harpenden, who have co-operated with us in these investigations.

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EXPLANATION OF PLATE 6

Fig. 1. Tree dying in the spring of 1958 from holdover canker in the trunk. This tree was first infected in 1957 in two branches, both of which were cut off during the winter 1957–8.

Fig. 2. Left, fruitlet of Laxton's Superb, 5 days after infection with *E. amylovora*, showing blackening lesion and bacterial ooze. Right, control fruit. (Natural size.)

Fig. 3. Shoot of Williams's Bon Chrétien pear, 5 days after inoculation with *E. amylovora*. Note scattered beads of bacterial ooze, and advance of lesion into petiole (top left). ($\times \frac{1}{2}$.)

Fig. 4. Blossom of Conference pear, 7 days after infection with *E. amylovora*, showing withered stamens, water-soaked petioles and surface bacterial ooze. Note fruitlet beginning to swell in the lowest, uninfected, blossom. ($\times \frac{3}{4}$.)

Fig. 5. Field tree of Laxton's Superb pear 6 weeks after inoculation of branches with *E. amylovora*. Extreme left, uninoculated control branch.

(*Received* 23 *December* 1959)

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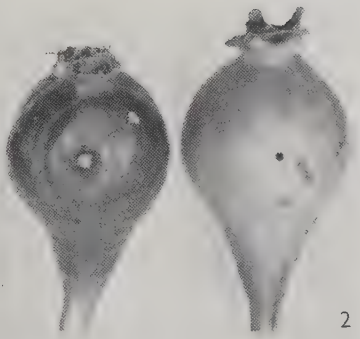
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CROSSE AND OTHERS—*Investigation of fire-blight of pear in England*

(*Facing p.* 558)

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COMPARISON OF TWO BARLEY YELLOW-DWARF VIRUSES IN GLASSHOUSE AND FIELD EXPERIMENTS

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(With Plate 7 and 3 Text-figures)

Comparisons were made of the host ranges, interactions in infected plants, and effects on yields of cereals of two isolates of barley yellow-dwarf virus, one avirulent, RV, obtained from Rothamsted farm (Watson & Mulligan, 1956) and the other virulent, KV, obtained from Kent. They resembled each other and the American yellow-dwarf viruses in their ability to infect wild grasses but differences were found when the infection tests were made using inbred lines of the same grass species. KV infected one variety each of rice, rye and maize, and caused symptoms in each.

When plants were first infected with RV and later, when symptoms had fully developed, with KV, they suffered the same loss of yield from KV as did plants infected for the same length of time with KV alone. Therefore plants infected with RV were not protected against infection with KV. Similarly, aphids (*Rhopalosiphum padi* L.), fed first on sources of RV and then on KV, transmitted mainly KV so there was no evidence of protection in the insect vectors.

Effects on yield of cereals were related to the time of appearance and intensity of symptoms. The effect of RV was not only less than that of KV but was also more variable.

Introduction

An isolate of barley yellow-dwarf virus (Oswald & Houston, 1951; *Review of Applied Mycology* (*Supplement*) 1957), found in barley on Rothamsted farm in 1954 was described by Watson & Mulligan (1957) under the name, cereal yellow-dwarf virus, used by Oswald & Houston (1951). It caused yellowing and stunting in Plumage Archer barley, and was transmitted by *Rhopalosiphum padi*, *Sitobium fragariae* (Walker), *Metopolophium dirhodum* (Walker) and *Myzus circumflexus* Buckt. In a field test in 1955 it decreased the yield of Plumage Archer by 37% when plants were infected by *R. padi* 25 days after drilling, and by 19% when infected 32 days after drilling (Watson & Mulligan, 1957).

In 1956 (Watson, 1958) four varieties each of wheat, oats and barley were experimentally infected with this isolate on two dates, the first 30 days and the second 54 days after drilling. The effects on yield were variable. Some varieties of wheat and oats seemed to lose more when infected late than early, but the experimental errors were large. Barley varieties Proctor, Provost, Rika and Maythorpe lost consistently as a result of the earlier infection but not of the later one. Some of the losses of other cereals were significant, but different varieties behaved differently both in susceptibility and reactions to different infection dates.

In 1957, after the mild winter of 1956–7, oat crops in the south and west of England were found to be infected with barley yellow-dwarf virus (BYDV) and some were seriously damaged. Virus isolated by *R. padi* from some of these crops seemed in glasshouse tests to be much more virulent than that isolated at Rothamsted in 1954. Virulent isolates were also obtained from crops in Hampshire, Kent, Gloucestershire, Shropshire and Cardiganshire. The Kent isolate (KV) was selected for comparison with the avirulent Rothamsted virus (RV), to determine their relationship to each other, their host range in cereals and grasses, and their effects on yield of cereals.

METHODS

R. padi was always used as the vector. The aphids were mainly cultured on oats, which is a favourable host, but because of the danger that oats might become contaminated with BYDV, stocks were also maintained on *Arrhenatherum elatius*, which is immune, and the oat cultures renewed from these.

Blenda oat was used in the glasshouse as host for the virus. Six to eight plants were grown to a 6-in. pot, and inoculated at the stage when the first leaf was beginning to expand. To infect them virus-free aphids were fed 48 hr. on detached infected leaves showing symptoms. The leaves, with their cut ends plunged in damp sand, were enclosed in glass vessels with tops covered by cellophane. To inoculate the young plants the requisite number of aphids was placed directly on to the shoots which were individually enclosed in glass or celluloid tubes. For less critical work, the aphids were shaken on to folded strips of thin paper, which the seedlings were interlaced to support, so that the aphids could immediately crawl on to them. After 48 hr. test feeding, the aphids were killed by aqueous phosdrin spray and the plants kept in a light, heated glasshouse. Plants were recorded as infected when the second or third leaf became discoloured. In spring and summer this happened from about 12 to 17 days after inoculation with the Kent virus and a few days later with the Rothamsted one.

For field experiments crops were grown in as nearly as possible ordinary farm conditions. The aphids were fed on infected plants for about 1 week, and were then collected approximately in dozens in corked glass tubes. These were opened on the site of the experiment and about forty tubes placed along the rows of each subplot. The subplots were covered by muslin and cellulose nitrate cages covering four 2 ft. rows of cereal. Corresponding control areas were caged but not infested with aphids. In 1956 aphids from healthy oats were placed in the control cages, but had no effect on the appearance or yield of the plants. There were apparently too few aphids for their feeding to cause direct damage during the week they were allowed to remain on the plants. At the end of this week the plants under all cages were sprayed with nicotine and when all the treatments had been given, the whole area was sprayed with Metasystox. Systemic insecticide was not used earlier for fear of jeopardizing the later infections by spray drift.

Results

Host range

Oswald & Houston (1953) tested fifty-five species of Gramineae as hosts of the California yellow-dwarf virus. Twenty species produced recognizable symptoms and nineteen appeared to be immune. Another sixteen produced no symptoms but yielded virus, and were therefore classed as carriers. Bruehl & Toko (1957) tested eighty-four species for susceptibility to the two Washington yellow-dwarf

Table 1. *Host range of British and United States isolates of BYDV*

	Rothamsted		Washington			
	RV54	KV	Original	English	California	
Oryza sativa, GEB 24	.	++	.	.	.	—
Zea mays, White Horse Tooth	.	+	.	.	.	V X
Z. mays, U.S. var. (Golden × Bantam)	.	.	—	—	o	V X
Cynosurus cristatus L., Bg486	+	.	.	.	.	V
Dactylis glomerata L., S26, S37 and S143	.	+	.	.	.	V X
D. glomerata L., U.S. variety	.	.	+	++	+	V X
D. glomerata L., ten other U.S. varieties	.	.	o	o	.	V X
Festuca arundinacea Schreb., S170	.	+	+	+	+	V
F. ovina L., S58	.	+±	+	+	.	V
F. pratensis Huds., S53 and S215	+	+	.	.	.	V
F. rubra, S59	o	+	+	+	.	V X
Lolium multiflorum Lam., S22	+	+	.	.	.	V
L. multiflorum Lam., U.S. variety	.	.	.	.	+	V
L. perenne L., S23	+	.	.	+	.	V X
L. perenne L., S24	+	+	.	.	.	V X
L. perenne L., PRG12 (from U.S.A.)	o	o	.	.	.	V X
Lolium, U.S. varieties	.	.	o	++	.	V X
Poa annua L., Wild	+	+	.	.	+	W
P. pratensis L., S63	o	+	+	o	+	V X
P. trivialis L., BK322	o	.	.	.	.	V X
BK325	.	+	.	.	.	V X
Bromus arvensis L. (U.S. *mollis*), Wild	.	++	++	++	.	W
B. sterilis L., Wild	.	++	.	++	.	W
Agropyron repens L. (U.S. *cristatum*), Wild	o	o	o	o	o	W
Secale cereale L., King II	.	++	.	.	.	—
Anthoxanthum odoratum, Bs2117	.	+	.	.	+	V
Arrhenatherum elatius L., Bm174	o	o	.	o	o	V
Avena fatua L., Wild	++	++	++	++	++	W
Agrostis tenuis Sibth. (U.S. *alba*), Wild	o	.	o	.	o	W
A. tenuis Sibth., N.Z. Browntop, Br963	.	+	.	.	.	V X
A. tenuis Sibth., N.Z. Browntop, Br969	o	.	.	.	.	V X
Alopecurus agrestis L., Bs1868	+	+±	.	.	.	V
A. pratensis L., Bh522	o	+	.	o	o	V X
Phleum pratense L., S48, S50, S51	.	++	.	.	.	V X
Phleum pratense L., U.S. varieties	.	.	+	++	o	V X

+, Host infected but symptomless; +±, very faint symptoms or dwarfing; ++, obvious symptoms: o, no infection; . , not tested.

W, tested in naturally occurring (Wild) host, and tests agreeing between all viruses tested; V, tested in cultivated varieties and results agreeing; VX, tested in cultivated varieties and results disagreeing between viruses. 'S' varieties bred by the Welsh Plant Breeding Station, Aberystwyth; other reference numbers belong to the W.P.B.S. collection.

viruses, Original and English. Forty of these were the same as those tested by Oswald & Houston. In nine species the California virus differed from one or other or both of the Washington viruses: the most important of these were *Bromus inermis*, which was immune to both Washington isolates but susceptible to the Californian, and timothy (*Phleum pratense*) which was the reverse. In nine species the two Washington viruses differed from each other, the most interesting being that the Original Washington virus and the Californian virus infected three out of three species of *Poa* tested, whereas the English Washington virus infected none out of five.

Table 1 compares the species of Gramineae, other than wheat, oats and barley, that have been tested with the Rothamsted and Kent viruses, compared with the American results.

The Kent virus infected rice, var. GEB 24, and caused symptoms similar to those in other cereals, and rye, var. King II in which it caused only slight stunting. The virus was recovered from these plants. Oswald & Houston tested a hybrid maize, Golden × Bantam, without result, but the Kent virus infected var. White Horse Tooth causing slight stunting, and was recovered from it. Rice and maize appear to be previously unrecorded hosts, and the susceptibility of some varieties of rice is of particular interest.

Bruehl & Toko noted differences in host range between the Washington and Californian isolates of BYDV. Similar discrepancies exist between these and the British isolates. However, when the results in Table 1 are grouped according to the origins of the seeds, the five different virus isolates differ in some respect nine out of seventeen times when tested in cultivated varieties, but agree in six tests of naturally occurring (wild) species of grasses. Possibly resistance to one or other of the viruses has been unconsciously selected for by breeders, or response to virus infection has been affected by selection for some other character. Differences at the generic level, for example the behaviour of the Washington viruses in the genus *Poa*, are probably genetically stable, but those between species cannot be depended upon as they may have been induced by more recent segregation of genes.

Relation between the Rothamsted and Kent isolates of BYDV

Behaviour when inoculated to the same plants

The two Washington isolates, Original and English, described by Toko & Bruehl in 1956, were distinguished by differences in symptoms, host range and vectors. The English isolate was transmissible only by the English grain aphid (*Macrosiphum granarium* Kirby), whereas the Original Washington isolate was transmissible by both the English grain and the apple grain aphid (*Rhopalosiphum fitchii* Sanders). These aphid identifications are open to question according to European systematists. When Toko & Bruehl inoculated the two viruses successively to the same plants, allowing sufficient time for the first to cause systemic symptoms, each virus was recovered by its specific vector when sufficient time had elapsed for the second to become systemic. Allen (1957) described similar results using isolates of varying virulence from the Californian virus.

KV and RV were only distinguishable at this time by virulence; they yielded results similar to those of the U.S. workers in two experiments, one made between February and April and the other between August and October. A group of young seedlings was infected with RV, using ten aphids per plant, and an equal number of seedlings were kept healthy. Four weeks later, when symptoms were fully developed, half the infected and half the healthy plants were infected with KV. The plants were harvested when about 10 weeks old, 4 weeks after the KV inoculation. Their fresh weights were recorded and their leaf area estimated from the product of length and breadth of leaves and sheaths, multiplied by a factor calculated from planimeter measurements of blue prints from random leaf samples.

TABLE 2. *Average weights and leaf and sheath areas for Blenda oats after being infected with RV, KV, and RV followed by KV*

		Infected with			
	Healthy	RV early	KV late	RV early + KV late	S.E.
Exp. I					
Weight of whole plant (g.)	8·10	4·62	2·48	2·84	*a* 0·166 *b* 0·338
Weight of tillers (g.)	3·29	0·49	0·21	0·12	*a* 0·215 *b* 0·319
Leaf and sheath area of whole plant (sq. dm.)	215·0	107·7	56·9	66·3	*a* 10·37 *b* 15·85
Exp. II					
Weight of whole plant (g.)	3·51	2·44	2·44	1·90	0·21
Leaf and sheath area of whole plant (sq. dm.)	109·2	88·6	54·8	50·9	5·23

a = For comparisons H *v.* KVL, RVE *v.* (RVE+KVL); *b* = for comparisons H *v.* RVE, KVL *v.* (RVE+KVL).

Table 2 shows that in Exp. I, the average total fresh weight of aerial parts of healthy plants, harvested 3 weeks after infection, was 8·1 g., of which 3·3 g. was the weight of tillers. Tillering was much reduced in RV infected plants and negligible in those infected with KV. In Exp. II the average weight of healthy plants was 3·5 g., and none produced tillers, so infection and autumn conditions seem to have had similar effects in suppressing development of side shoots. Both, presumably, limited the amount of carbohydrate supplied to growing tissues, and this may have affected shoot production.

The losses of fresh weight caused by the three treatments, RV inoculated early, KV inoculated late, and RV-early followed by KV-late were respectively 43, 69 and 65% in Exp. I; 35, 35 and 46% in Exp. II. In both experiments KV caused as great a loss from plants already infected with RV as it did from hitherto uninfected plants, so there was no indication that previous RV infection interfered with the effect of KV (Pl. 7, fig. 1).

The losses caused by RV and KV are not directly comparable because the experiments were arranged so that they were applied at different times, but there was

less difference between them, and a smaller proportional loss of mean yield caused by infection in Exp. II than in Exp. I, presumably another consequence of less daylight in the autumn experiment. Similar effects were observed with beet yellows virus, which in a field experiment (Watson, 1942) reduced the yield of exposed plants much more than that of shaded ones. The external symptoms of yellow dwarf and beet yellows are also similar, and both cause starch to accumulate in leaves showing symptoms (Watson & Watson (1951) and Pl. 7, fig. 2).

TABLE 3. *Average heights of plants infected with isolates from plants in Exp. II*
(Measured in inches, base to uppermost ligule; three plants inoculated from each source.)

	Independent sources of virus				
	1	2	3	4	S.E.
	Heights of plants (in.)				
Virus from (RVE+KVL)	6·7	4·7	10·3	6·3	0·53
Virus from RVE	12·5	10·7	12·2	11·8	

Table 3 shows the average heights of plants 45 days after they were colonized as seedlings by aphids that had fed on the four groups of plants in Exp. II. The four sources of RV and (RV+KV) were compared in opposite halves of the same pots of six seedlings, and four pots were used for each source. The average height of plants inoculated with virus from plants infected first with RV and then with KV was 7 in. and that of plants inoculated with RV alone, 11·7 in. There was a significant interaction between samples from the two sources which is almost wholly accounted for by the one tall average (10·3 in.) group of plants among those inoculated from the doubly infected plants. Possibly this sample contained RV and the others KV, both viruses having survived independently in the same source plant.

Behaviour when acquired by the same aphids

In another experiment *R. padi* was made to feed first on plants infected with RV and then on plants infected with KV, to test whether there is any interaction between the viruses in the aphids. The aphids were allowed to feed for 48 hr. on each source separately, and also on RV first and then on KV. They were then placed, ten per seedling, for 48 hr. on test plants. Treatments (RV+KV) and RV were compared together within sixteen pots of six to eight plants. KV and RV were separately compared in eight more pots. Infected plants were not measured but the symptoms were rated as: mild, moderate, severe and very severe.

Table 4 shows that RV infected plants developed only mild symptoms. KV and the successively fed aphids caused mainly severe or very severe symptoms, but the (RV+KV) infected group contained a few more plants with moderate or mild symptoms than did the group infected with KV alone. Apparently the aphids that fed last on KV introduced KV into most of the healthy plants they fed on. They must have been fully infective with RV when they acquired the second virus, but it became the dominant one, probably the only one inoculated to most of the test plants.

As in the previous experiments virus-free aphids were fed on the differently

infected plants, and isolates taken to fresh healthy seedlings, which were measured for height about 4 weeks after infection.

Table 5 shows that the amount of stunting of the plants infected with isolates derived indirectly from the twice-fed aphids was about what would be expected for KV, and considerably more than RV alone would have caused. However, as in previous tests with mixed infections, there was more variation in height of (RV + KV) infected plants than of those infected from the pure source. Possibly some aphids had acquired both viruses, from doubly infected plants, or there may have been some interaction between the viruses in the plants from which they were derived, to account for the variability.

TABLE 4. *Symptom ratings of plants infected with RV and KV separately and with RV followed by KV*

Viruses	Numbers of plants tested	Numbers in different symptom groups				
		Mild	Moderate	Severe	Very severe	Total
RV	64	38	0	0	0	38
KV	44	0	2	16	18	36
RV followed by KV	48	1	4	16	22	43

TABLE 5. *Average heights (in.) of plants infected with virus from plants infected by* Rhopalosiphum padi *that fed for 2 days on RV and then 2 days on KV, with RV controls*

(Three plants inoculated from each source.)

Isolates from plants infected by	Independent sources of virus								S.E.
	1	2	3	4	5	6	7	8	
	Heights of plants								
Aphids fed only on RV	10·8	10·0	8·7	8·5	11·0	10·0	9·9	9·7	0·47
Aphids fed on RV followed by KV	6·0	8·3	6·3	4·5	5·8	5·7	5·0	7·0	

Effects of RV and KV on yields of wheat, oats and barley

Field experiments were made from 1955 to 1957 to test the effect of RV on cereals infected at different times throughout the growing season. In 1958 RV and KV were compared. The results for 1955 and 1956 have been reported elsewhere (Watson & Mulligan, 1957; Watson, 1958) and are reviewed in the introduction to this paper. The present account includes results for 1957 and 1958 only.

The arrangement in 1957 was of eighteen plots in three blocks of six cereal varieties, two each of wheat, oats and barley. Each plot was split for three infection dates and healthy control. In 1958 there were three randomized blocks of three plots for one variety of each cereal (Koga II wheat, Proctor barley, Blenda oat), that had been used in 1957. The plots were split for three infection dates with each of the two viruses, KV and RV, and two healthy controls, making eight subplots.

Table 6 gives dates of observations and treatments. As the sowing dates differed

TABLE 6. *Dates (with numbers of days after drilling) of operations and observations on field plots*

Observations on healthy plants	1957 experiment					1958 experiment			
	Date	Day number	Variety	Plant count*	Tiller count (%)†	Date	Day number	Plant count*	Tiller count (%)†
Drilled	18. iii	1	—	—	—	25. iii	1	—	—
Braird	2. iv	16	—	—	—	17. iv.	24	—	—
Tiller and plant counts									
1	—	—	—	—	—	25. iv.	31	—	No tillers
2	25. iv.	39	Koga	43	14	2. v.	39	—	Rare
			Blenda	40	6			—	Rare
			Proctor	32	100			—	Many
3	3. v.	47	Koga	40	68	8. v.	44	50	81
			Blenda	38	61			58	66
			Proctor	32	100			30	98
4	15. v.	59	Koga	—	2·2‡	22. v.	58	50	2·3‡
			Blenda	—	2·0‡			59	1·8‡
			Proctor	—	4·8‡			38	3·2‡
Ears emerging	18. vi.	(K) 93	—	—	—	24. vi.	91		
	20. vi.	(B) 95	—	—	—	25. vi.	92		
	21. vi.	(P) 96	—	—	—	25. vi.	92		
Infection date									
1	24. iv.	38	—	—	—	2. v.	38		
2	4. v.	48	—	—	—	16. v.	54		
3	14. v.	58	—	—	—	2. vi.	70		

* Plants per 1 yard length of row.
† Proportion of plants tillered, or no. of tillers per plant.
‡ Per plant.

TABLE 7. *Effect of different dates of experimental infection with RV and KV on yields of grain and straw (air-dried) from field plots of wheat, oats and barley*

Virus infection	RV 1957						RV 1958			KV 1958		
	Atle	Milford	Herta	Koga	Blenda	Proctor	Koga	Blenda	Proctor	Koga	Blenda	Proctor
	Yields of grain (cwt per acre)											
None	36	26	39	35	34	40	40	34	36	39	30	36
Late	37	22	41	38	27	34	36	36	36	24	29	33
Middle	34	21	41	34	25	41	26	18	31	22	16	24
Early	33	21	38	37	22	33	24	15	30	14	6	14
S.E.												
Across*	1·73	1·50	1·51	1·73	1·50	1·51	2·23	2·41	2·45	2·23	2·41	2·45
Downwards	1·73	1·73	2·06	1·73	1·73	2·06	3·15	1·70	3·46	3·15	1·70	3·46
	Yields of straw (cwt per acre)											
None	58	64	55	65	53	55	67	57	66	70	52	62
Late	56	54	55	66	46	51	69	68	72	68	61	67
Middle	56	58	58	66	47	55	68	49	59	59	54	61
Early	61	51	55	66	40	48	58	55	61	43	29	40
S.E.												
Across*	3·44	3·14	2·40	3·44	3·14	2·40	3·15	2·68	3·98	3·15	2·68	3·98
Downwards	2·98	3·43	1·95	2·98	3·43	1·95	2·23	1·89	2·81	2·23	1·89	2·81

LSD 5 % = 2·15; 1 % = 3·00.

* Appropriate for different varieties of the same cereal in 1957, different viruses in 1958.

in the two years these times are also expressed as numbers of days after drilling. Plants and tillers were counted in random samples of three or six paired 1-yard lengths in non-experimental parts of the plots.

Differences in effects of RV and KV

Table 7 compares the yields of grain and straw for plots treated at different times and for uninfected plots. In 1958 KV caused greater loss than did RV in either year. Grain yields were much reduced by the first two infections. The first brought the yield of oats almost to zero, for the result reflected mainly the yield of a few plants that either resisted infection or escaped being fed on by infective aphids during the first week.

The earlier infections with KV caused much stunting, which affected the straw yields, but on the whole the loss was less than of grain. RV caused little loss of straw, as in previous years, except for oats in 1957.

Text-fig. 1 shows the mean yields for all cereals plotted against time of infection. The curve for KV suggests that the effect of this virus was simply related to the age of the plant hosts at the time of infection, whereas with RV the first and second dates caused similar losses and the third none. Thus the effect of RV seemed to be more affected by extraneous factors than that of KV, and these factors presumably account for the variations in results in the previous three experiments.

The mean loss caused by KV at the third time is rather overweighted by the large loss from Koga II wheat, which was harvested later than the other cereals and possibly suffered more through loss of assimilate from flag leaves and glumes. In general, yields seem to be little affected when plants become infected fewer than about 6 weeks before harvest, but earlier infection may cause very severe losses.

Differences between years in effects of RV

The first infection was made 38 days after drilling in both years, but the later ones were 10 days apart in 1957 and 16 days in 1958. It is the more remarkable that the second infection (with RV) reduced yields more in 1958 than in 1957.

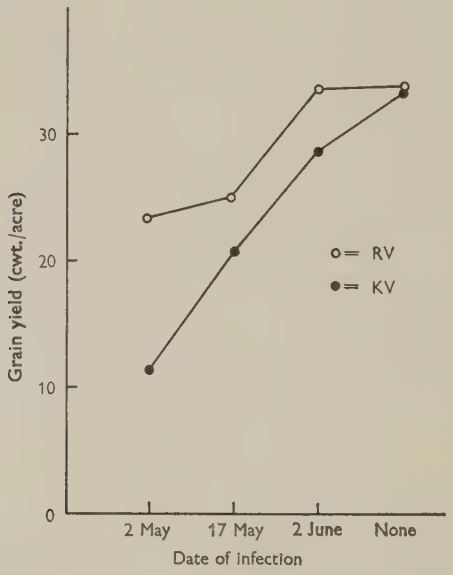
There was no evidence that the stage of development of tillers when the plants became infected was critical to the effect of infection. The results differed widely in the two years, although infection was applied at about the same stage of tillering. We attempted to obtain more exact information about the relation of time of infection to tillering by ringing plants of the 1957 experiment at infection time to mark the leading shoot. The ears from leading and side shoots of these plants were weighed separately at harvest. The data were not sufficiently accurate to assess individual losses, but tillering was not completely suppressed at any particular time. Even in infected plants tillers of wheat and barley seemed to contribute a more or less constant proportion of the total yield, and produced ears about as frequently as the leading shoots. Only in Blenda oats were tillers significantly suppressed. They were suppressed at all infection times but mainly at the first, when tillering was less well advanced than in the other cereals. In Blenda oat the

TABLE 8. *Symptom scores, totals for three plots (lowest 0, highest 7), scored on different dates in 1957 and 1958*

	No. of days between sowing and infection	Dates of observation of symptoms: 1957 RV 14. v.*	29. v.	5. vi.	15. vi.	26. vi.†	1958 RV 22. v.*	5. vi.	13. vi.	23. vi.	7. vii.†	1958 KV 22. v.	5. vi.	13. vi.	23. vi.	7. vii.
Koga II	38	0	0	2	4	7	4	7	11	11	14	6	9	13	15	21
Wheat	48	0	0	0	2	5	—	—	—	—	—	—	—	—	—	—
	58–54‡	0	0	0	0	1	0	0	4	11	13	0	0	10	12	14
	70	—	—	—	—	—	0	0	0	2	3	0	0	0	0	6
	None	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0
Blenda	38	0	6	11	12	15	5	8	11	15	17	12	15	15	18	21
Oat	48	1	0	2	9	12	—	—	—	—	—	—	—	—	—	—
	58–54‡	0	0	0	2	5	0	0	3	8	15	0	0	6	12	15
	70	—	—	—	—	—	0	0	0	0	1	0	0	0	1	4
	None	0	0	1	2	3	0	0	0	0	0	0	0	0	0	2
Proctor	38	2	7	9	12	12	4	7	7	11	7	9	14	15	14	17
Barley	48	0	1	0	2	3	—	—	—	—	—	—	—	—	—	—
	58–54‡	0	0	0	2	2	0	0	2	6	8	0	0	7	13	11
	70	—	—	—	—	—	0	0	0	0	2	0	0	0	0	6
	None	0	0	1	1	2	0	0	0	0	0	0	0	0	0	0

* 20 days after first infection day in 1957 and 1958.
† 63 days after first infection day in 1957, 66 in 1958.
‡ 58 days in 1957, 54 days in 1958.

main loss of yield was from the tillers, in other cereals, Proctor barley, for instance, it was equally from the main shoots. These results may indicate that cereals and varieties that tiller late may suffer more from infection with BYD viruses than those which tiller early, but the main variations in losses caused by infection do not seem to be explained.



Text-fig. 1. Average yields (means of wheat, oats and barley) of cereals infected on different dates with RV and KV.

The relation of symptoms of loss of yield

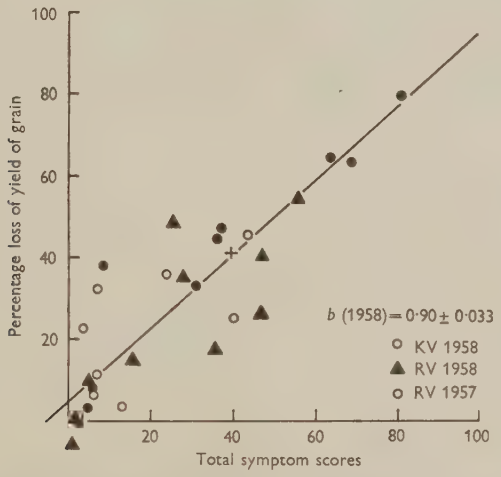
Individual plants cannot be distinguished after the first few weeks of growth of a cereal crop, so infected plants could not be counted, but the plots were rated for symptoms roughly according to the following scale:

(1) Yellow or red discoloration (chlorosis) just visible.
(2) Chlorosis visible without parting plants.
(3) Chlorosis obvious and affecting several leaves.
(4) Chlorosis conspicuous at a distance.
(5) Conspicuous chlorosis accompanied by reduction in height (measured with a rod suspended into the plot).
(6) Conspicuous chlorosis, necrosis and severe stunting.
(7) Conspicuous chlorosis, necrosis and very severe stunting.

Table 8 shows the symptom ratings given to different plots on the same day, in the two experiments, comparing the same cereal varieties.

Symptoms developed at different rates in the two years, for plants infected 54 days after drilling in 1958 received higher symptom ratings than those infected

48 days after drilling in 1957. Another difference between years was that, in 1957, some plants not experimentally infected developed symptoms, presumably as a result of natural introduction of virus. This probably occurred during the week that the plots experimentally infected on the third occasion were caged, and possibly the virus was more virulent than ours, because symptoms on uninfected parts of the experiment appeared before those of the third infection. There were only a few of these compared with the numbers that eventually developed on the experimentally infected plots, but they may have decreased the difference between yields of healthy and infected plots.



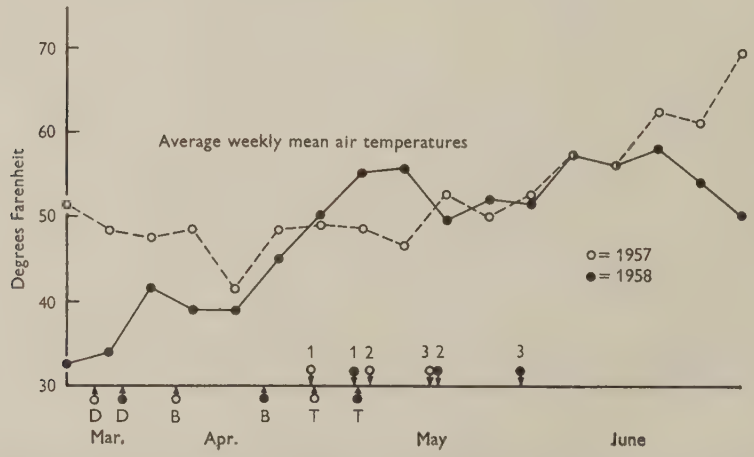
Text-fig. 2. Relation between symptom ratings and percentage loss of yield of cereals infected with RV in 1957, RV and KV in 1958. The regression coefficient was calculated for the 1958 data and the 1957 results adjusted accordingly.

Text-fig. 2 shows the relation between symptoms (total scores for all observation times), and percentage decrease in yield for both viruses. For the 1958 experiment the points fall reasonably near to a straight line with a correlation coefficient $b = 0{\cdot}90 \pm 0{\cdot}033$. Thus yield fell by about 1 % for every mark given in the total rating for three plots scored on five occasions. The RV results are more scattered than those for KV, but appear to be similarly related, and the difference between the way the two viruses affect yield seems to be well reflected in the visible symptoms they cause.

The results for 1957 were adjusted by adding 1 % to the yield of the control plots for each mark given in the symptom ratings for naturally introduced infection. The regression coefficient was increased from $0{\cdot}28 \pm 0{\cdot}310$ to $0{\cdot}67 \pm 0{\cdot}272$, and the points now fall about the line calculated for 1958, whereas the uncorrected points fell below it.

DISCUSSION

Giddings (1950) showed that viruses which he held to be strains of curly top virus did not protect against each other in sugar beet. Bennett (1955) suggested that this was because the viruses were readily adsorbed or aggregated in the plant tissues. Similarly, the 'mild yellowing viruses' (Watson, 1951) of sugar beet do not protect against beet yellows virus, although they closely resemble it in properties and in relationship with the aphid vectors. No antiserum against the barley yellow-dwarf viruses has yet been obtained, despite repeated attempts, so there are at present no other criteria on which to decide their relationships. One suggestion might be that the lack of antibody formation, the obligate nature of the vector transmission, and the absence of the cross-immunity reaction may all have the same cause, namely lack or scarcity of protein in the virus particles, and that such viruses can be strains without giving the accepted reactions.



Text-fig. 3. Weekly averages of mean air temperatures in 1957 and 1958 showing their relation to seed emergence, tillering and date of infection, with RV and KV. Arrow indicators below abscissa: D = date drilled; B = date brairded; T = date tillered. Arrow indicators above abscissa 1, 2 and 3 = 1st, 2nd and 3rd infection date.

Barley yellow dwarf is typical of the aphid-transmitted yellowing diseases of crop plants that injure the hosts by interfering with carbohydrate metabolism or translocation. Other examples are sugar-beet yellows and leaf-roll virus of potato. Leaves of sugar beet that are yellowed as a result of virus infection accumulate large quantities of carbohydrate in the form of starch sucrose and reducing sugars (Watson & Watson, 1951). Chlorotic leaves of cereals infected with RV and KV also contain high concentrations of reducing sugars, although, at least in the glasshouse, the increases are much smaller than those in sugar beet. Cereals do not normally accumulate starch in their leaves (Archbold, 1940), but leaves of wheat

(Pl. 7, fig. 2) infected in the field showed conspicuous starch formation when decolorized and stained with iodine.

This behaviour possibly gives a clue to the cause of variability in the effects of the virus on yield. In the glasshouse we found that yields of leaves and stems seemed less affected in autumn than in spring. In the field symptoms developed more slowly and the loss of yield was less in 1957 than in 1958. As 1958 is memorable as a particularly wet summer, this seems contradictory, but Text-fig. 3 compares the weekly averages of daily temperatures (mean of maximum and minimum), covering the times of drilling and experimental infection. In 1958 cold weather up to the end of April delayed germination, and although the infections were made a week later to allow for the later drilling the time between germination and infection was shorter than in 1957. Furthermore, although the season as a whole was cold and wet, there were warm spells in May and June coinciding with the periods when symptoms were developing after the experimental infections, whereas in 1957 the corresponding periods were cold and wet. With sugar beet (Watson, Watson & Hull, 1946), the time of appearance of symptoms of yellows virus was directly related to loss of yield. This seems to be true also of BYDV, and because external conditions, particularly light and temperature, can delay or accelerate symptom development, loss of yield is more predictable from these than on the actual time of infection.

RV may behave more erratically than KV because it multiplies more slowly in the plants, and small or brief changes in the environment at critical stages of growth, or in the responses of particular varieties to these changes, have greater effect.

We gratefully acknowledge the help and co-operation of Mrs Elizabeth Lennon and Dr D. A. Govier of the Scottish Plant Breeding Institute, and of Mr J. H. A. Dunwoody with the statistics. We also thank the Welsh Plant Breeding Institute for seed of grass varieties, the National Institute of Agricultural Botany and the Plant Breeding Institute, Cambridge, for seed of cereal varieties. The photograph was taken by Mr F. Cowland.

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EXPLANATION OF PLATE 7

Fig. 1. On right: plants of Blenda oat infected when 1 week old with RV, photographed at 9 weeks. On left: plants infected at 1 week old with RV and at 5 weeks old with KV, also photographed at 9 weeks. The stunting caused by KV is about the same as would be expected had no RV inoculation been given.

Fig. 2. Accumulation of starch in leaves of Capelle wheat infected with BYDV, shown by iodine staining after decolorizing in alcohol. Left, healthy; right, infected.

(*Received* 14 *January* 1960)

Fig. 1

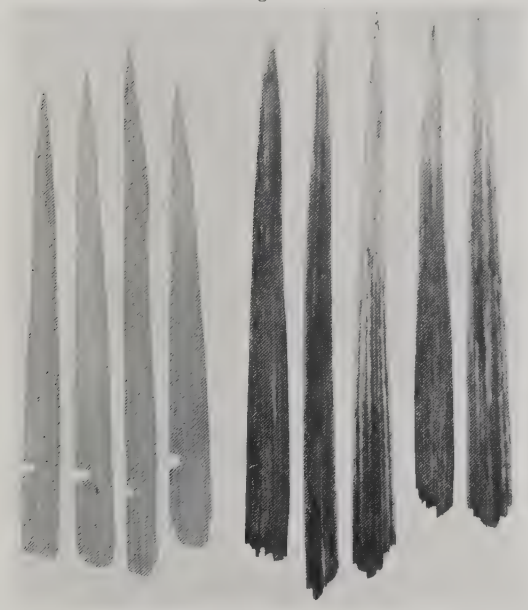

Fig. 2

WATSON AND MULLIGAN—*Comparison of two barley yellow-dwarf viruses*

(*Facing p.* 574)

Mulligan, T. E. (1960). *Ann. appl. Biol.* **48** (3), 575–579

THE TRANSMISSION BY MITES, HOST-RANGE AND PROPERTIES OF RYEGRASS MOSAIC VIRUS

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(With Plate 8)

A virus that causes chlorotic streaks on ryegrass leaves was transmitted by the eriophyid mite *Abacarus hystrix* (Nalepa). Virus-free mites acquired the virus in 2 hr. feeding on infected ryegrass and the proportion that became infective increased with increased feeding time up to 12 hr.; vectors lost infectivity within 24 hr. of leaving the infected leaves. All instars of *A. hystrix* transmitted the virus.

The virus was transmitted by manual inoculation of sap to other species of Gramineae, including oats, rice, cocksfoot and meadow fescue, but none of these hosts seemed to contain as much virus as ryegrass; their saps did not precipitate specifically with antiserum prepared against the virus in ryegrass, whereas sap from infected ryegrass precipitated up to a dilution of 1/32. Infective sap of S22 Italian ryegrass contained flexuous rod-shaped particles; the dilution end-point of the virus was about 1 in 1000; the virus was inactivated when held for 10 min. at 60° C. and most of its infectivity was lost after 24 hr. at room temperature.

Introduction

A virus causing pale-green streaks in ryegrass leaves was found in Britain by Dr J. T. Slykhuis, who transmitted it by sap inoculation and less readily by a mixed colony of eriophyid mites. He noted the disease in six other European countries and refers to it in his survey as 'ryegrass streak mosaic' (Slykhuis, 1958). Samples of mite colonies found on diseased ryegrass leaves in England were sent to Dr H. H. Keifer who identified *Abacarus hystrix* (Nalepa) in all four samples, *Vasates dubius* (Nalepa) in two and a species of *Eriophyes* in one of them.

Materials and methods

S22 Italian ryegrass gave the most distinct symptoms and was the plant most readily infected when inoculated with sap containing 'Celite' (Superfloss grade). Plants, ten in 6 in. pots, were inoculated at the 3- or 4-leaf stage. The properties of the virus were difficult to determine accurately because there is no plant that gives countable local lesions, and both the susceptibility of the test plants and the infectivity of the sources of virus varied.

Several isolates of the virus were obtained by collecting infected ryegrasses from the field and inoculating their saps to S22 Italian ryegrass. Isolates differed in virulence, some giving mild symptoms difficult to detect and others causing severe chlorotic and necrotic streaking. The most virulent isolate was adopted for

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use in experiments. In summer, plants showed symptoms within 7–10 days of infection; in winter, symptoms were less severe and developed more slowly.

The eriophyid mites live on the grooved upper surface of ryegrass leaves. They measure about $200 \times 50\,\mu$ and were transferred by a single hair glued to a wooden holder, as described by Slykhuis (1953), ten mites to each of five S 22 Italian ryegrass seedlings at the 1-leaf stage. The seedlings were covered by a cylinder of transparent Xylonite sheeting $\frac{1}{100}$ in. gauge (BX Plastics Ltd., London) with Nylon gauze sealed to one side for ventilation and Cellophane at the top.

Mites were fed on detached infected leaves of Italian ryegrass plants that had been showing symptoms of infection for about 5 weeks; few transmissions occurred when the first leaves to develop symptoms were used as source. The detached leaves were kept moist on damp filter-paper in Petri dishes.

Single mites were maintained on seedlings of S 22 Italian ryegrass grown separately in sterilized soil in large boiling tubes capped with Cellophane. Plants with colonies were transplanted later to 6 in. pots and kept covered.

Experimental results

Isolation of vector. Mites collected from infected plants in the field were transferred to seedlings of S 22 Italian ryegrass. The mites on plants that became infected were allowed to multiply and later were fed for an additional 2 days on detached infected leaves. After repeated tests, three cultures derived from single mites transmitted the virus and were identified as *Abacarus hystrix* (Nalepa). This species, like many other Eriophyids (Keifer, 1952), can reproduce by eggs without fertilization.

Factors affecting transmission by Abacarus hystrix. To find the length of feeding time on infected leaves necessary for mites to acquire virus, 500 virus-free *A. hystrix*, second instars and adults, were placed on infected leaves of S 22 Italian ryegrass and 100 mites were moved to ten healthy seedlings after they had fed for 6, 12, 24 and 48 hr. respectively. This experiment was repeated three times; 10% of the test plants became infected when the mites fed for 12 hr. or longer on the source of infection. However, in a similar experiment in which mites were fed 1, 2, 4 and 8 hr. on infected leaves, transmission occurred after they had fed for 2 hr. only (Table 1), and it is possible that even this does not represent the minimum time. In an experiment with different instars all transmitted equally often.

The period for which mites remain infective was found by feeding 600 virus-free mites on infected leaves of S 22 Italian ryegrass for 72 hr. and then transferring them to leaves of Cappelle wheat, which is immune to the virus and on which they feed readily. One hundred mites were moved to ten ryegrass seedlings after spending 3, 6, 12, 24 and 48 hr. on the wheat. On three occasions, the disease was transmitted after 6 hr. on the wheat but not after 12 hr. (Table 1, part B).

Properties of ryegrass mosaic. Freshly extracted sap of infected S 22 Italian ryegrass was used in all experiments.

Electron-microscopy of sap clarified by centrifuging at 9000 *g* for 10 min. showed flexuous rods measuring about 400×20 mμ, which do not occur in virus-free ryegrass and are presumably the virus particles. An antiserum prepared by in-

jecting a rabbit with clarified infective sap precipitated specifically with infective sap; the precipitate was of the flagellar type usually given by rod-shaped particles. The dilution end-point seemed to be about 1/1000 of sap diluted in water or phosphate buffer. Sap diluted 1 in 4 in phosphate buffer at pH 7 and held at different temperatures for 10 min. lost most of its infectivity between 40° and 55° C. and was inactivated at 60° C.

TABLE 1. *Infectivity of mites after feeding for varying times on infected plants (part A). Infectivity of mites kept for varying times on non-susceptible wheat after feeding on infected leaves for 72 hr (part B).*

(Numbers of ryegrass seedlings infected out of thirty tested, ten mites per seedling.)

	Times of feeding (hr.)										
	0	1	2	3	4	6	8	12	24	48	Untreated
Part A											
Feeding on infected plants*	0	—	—	—	—	0	—	3	3	3	—
	0	0	1	—	1	—	4	—	6	—	0
Part B											
Intermediate feeding on non-susceptible host*	10	—	—	5	—	3	—	1	0	0	—

Four equal samples of sap diluted 1 in 3 in water, phosphate buffer pH 7, 0·01 M sodium sulphite in phosphate buffer and 0·01 M cystein in phosphate buffer respectively were held at room temperature and tested at intervals of 2, 4, 8 and 20 hr. by inoculation to ten S 22 Italian ryegrasses on three occasions. All samples lost infectivity during 20 hr. When the sap was diluted with water the loss was from 73 to 10% and when it was diluted in phosphate, from 84 to 22%; the addition of cystein and sulphite seemed to increase the longevity slightly but this increase was within the limits of experimental error.

Host-range and symptoms. Sap from infected S 22 Italian ryegrass was inoculated with 'Celite' to many common British grasses and cereals. At least twenty-five plants of each species were inoculated and after 4–6 weeks sap was taken from these plants whether they showed symptoms or not and inoculated to S 22 Italian ryegrass (Table 2). This test confirmed the presence of the virus in most of the species that had chlorotic streaks, but no virus was recovered from *Bromus inermis* Leyss. and *Agrostis tenuis* Sibth., although they showed chlorotic streaks. Symptoms in infected *Bromus sterilis* L. were slight and transient.

Oats infected by manual inoculation developed pale chlorotic streaks that became 'rusty' on the upper leaves and on the palea; they were stunted and the lower spikelets were sterile. *Abacarus hystrix* transmitted the virus to Blenda oats causing similar symptoms; healthy mites caused no symptoms and did not multiply well on oats in the glasshouse. The mites multiplied readily on Proctor barley and Cappelle wheat but whether virus-free or infective they caused no symptoms. Timothy seemed to be immune and mites did not multiply on it or cause symptoms, though the same species has caused severe damage to Timothy crops in New Jersey (Pepper, 1942).

The streaking caused by ryegrass mosaic virus in S 143, S 37 and S 26 cocksfoot resembled symptoms caused by cocksfoot streak virus (Smith, 1952) but was more transient. Cocksfoot streak virus kindly sent by Dr K. M. Smith was transmitted to these three cocksfoot varieties and to S 22 Italian ryegrass by manual inoculation and by *Myzus persicae*. In Italian ryegrass both viruses produced similar symptoms but the cocksfoot streak virus was not transmitted by *Abacarus hystrix* and did not react with antiserum to ryegrass mosaic virus. This indicates that the two are unrelated.

TABLE 2. *The susceptibility of species of Gramineae to ryegrass mosaic*

Species tested	Variety*	Exp. A†	Other tests	Confirmatory tests in S 22
Festuceae				
Cynosurus cristatus L.	Bg 486	14/100‡ S.T. 0	3/5‡	18/20‡
Dactylis glomerata L.	S 143, S 37, S 26	—	3/74	13/117
Festuca pratensis Huds.	S 215	12/100 S.T. 0	2/5	18/20
Lolium multiflorum Lam.	S 22	69/100 S.T. 1/64	—	—
L. perenne L.	S 24	45/100 S.T. 1/32	—	—
Poa annua L.	—	21/100 S.T. 0	—	—
P. pratensis L.	S 63	—	0/39	1/40
P. trivialis L.	Bk 322	—	0/46	1/40
Brachypodieae				
Bromus arvensis L.	—	43/100 S.T. 0	—	5/20
B. inermis Leyss.	—	—	15/40	0/40
B. sterilis L.	—	/100	/6	12/39
Agrosteae				
Agrostis tenuis Sibth.	Br 963	—	8/50	0/40
Alopecurus agrestis L.	—	—	18/46	35/40
Aveneae				
Avena fatua L.	—	40/100 S.T. 0	2/10	1/10
A. sativa L.	Blenda, Milford	36/100 S.T. 0	3/30	4/20
Oryzeae				
Oryza sativa	GEB 24	—	1/16	9/10

* 'S' varieties bred by the Welsh Plant Breeding Station, Aberystwyth; other reference numbers belong to the W.P.B.S. collection.

† 'Experiment A': plants infected by same inoculum at 2–3 leaf stage; S.T. = serological titre of extracted sap.

‡ Number of plants infected/number tested.

The following species and varieties failed to become infected and confirmatory tests in S 22 were negative: *Festuca arundinacea* (Bn 229), 0/24, (S 170), 0/83; *F. ovina* (S 58), 0/55; *F. rubra* (S 59), 0/25; *Agropyrum repens*, 0/20; *Hordeum vulgare* (Earl, Kenia), 0/46; *Secale cereale* (King II), 0/45; *Triticum vulgare* (Bersee, Koga II, Fylgia, Cappelle), 0/68; *Alopecurus pratensis* (S 55), 0/55; *Phleum pratense* (S 48, S 50, S 51), 0/116; *Anthoxanthum odoratum*, 0/37; *Arrhenatherum elatius*, 0/40; *Holcus lanatus*, 0/55; *Zea mays* (White Horse Tooth), 0/34.

Many species of dicotyledons including those species often used for local-lesion assays of viruses were tested by sap inoculation with ryegrass mosaic, but none gave evidence of local or systemic infection. So far only the Gramineae have been found to be susceptible.

Serology. An antiserum was prepared by injecting a rabbit intravenously with the material sedimented from clarified sap of infected S 22 Italian ryegrass by centrifuging at 50,000 *g* for 1½ hr. Antiserum precipitated up to a dilution of 1/500

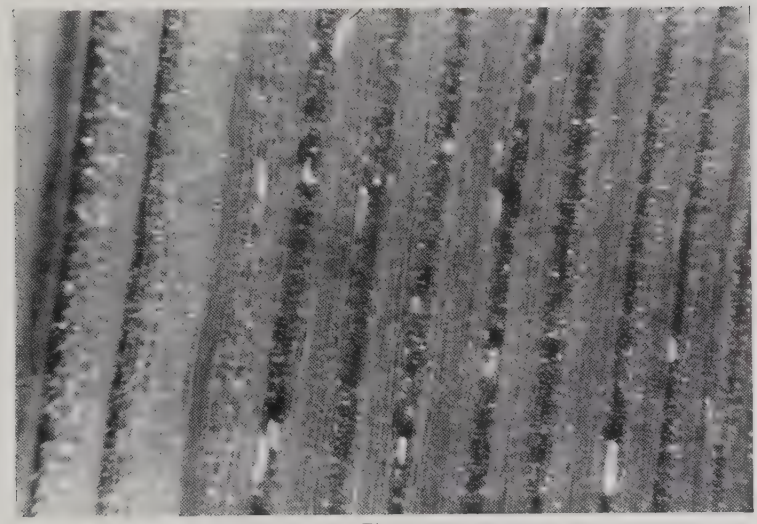

Fig. 1

Fig. 2

MULLIGAN—*Ryegrass mosaic virus transmitted by mites*

(*Facing p.* 578)

with sap from infected S22 Italian ryegrass. The antiserum at 1/100 titrated against sap from infected S22 Italian ryegrass reacted up to 1/64 and with infected S24 perennial ryegrass to 1/32.

Discussion

It is difficult to estimate the economic importance of this virus in the field. In the glasshouse, the fresh weight of infected S22 Italian ryegrass 3 weeks after infection was only two-thirds that of uninfected plants in the same pots. Reduction in size and vigour, apparently caused by infection, can be seen in single isolates in ryegrass variety trials and it probably reduces the yield of perennial ryegrass leys.

Infected plants and eriophyid mites have been found in ryegrass leys after only 4 or 5 months' growth, so that disease seems to enter the crop fairly quickly. The mites have been found in frosty weather and are probably wind-borne like *Aceria tulipae*, the vector of wheat streak-mosaic virus in the U.S.A. (Slykhuis, 1953; Pady, 1955). As the conditions favouring severe outbreaks of wheat streak in American crops are not understood (Bruehl & Keifer, 1958) it is not possible to predict the likelihood of outbreaks of ryegrass mosaic in Britain. A possible outbreak occurred in a winter oat crop in Berkshire. Sap from plants severely damaged by a necrotic streaking disease was inoculated to S22 Italian ryegrass which developed symptoms like those of a mild strain of ryegrass mosaic.

Ryegrass mosaic virus differs in host-range from a mosaic of Italian ryegrass and cocksfoot in Washington (Bruehl, Toko & McKinney, 1957) the vector of which was not described.

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EXPLANATION OF PLATE 8

Fig. 1. Symptoms of ryegrass mosaic virus on S22 Italian ryegrass.

Fig. 2. All stages of mite, *Abacarus hystrix* (Nal.), on leaf of S22 Italian ryegrass.

(*Received* 18 *November* 1959)

Burt, P. E., Broadbent, L. and Heathcote, G. D. (1960). *Ann. appl. Biol.* **48** (3), 580–590

THE USE OF SOIL INSECTICIDES TO CONTROL POTATO APHIDS AND VIRUS DISEASES

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(With 1 Text-figure)

A replicated trial was done to find whether the insecticides Thimet and Rogor applied in the soil affected the spread of aphid-transmitted viruses from infected to healthy plants with potato crops. The insecticides were applied at planting as activated carbon formulations at rates equal in cost to three sprays with DDT emulsion at 2 lb. DDT per acre. The infected plants were removed (rogued) in late June.

Thimet applied along the furrows with the fertilizer, and Rogor applied in individual doses beneath each tuber, kept the plants free from aphids from a week after the plants emerged until early August. Thimet in individual doses was less effective but greatly decreased the aphid infestation. All treatments prevented or greatly decreased the spread of leaf-roll virus, but they only slightly decreased the spread of virus *Y*. No treatment damaged the plants or depressed yields significantly.

Tubers harvested from the plots treated with insecticides contained only very small quantities of the insecticides, but shoots from them, when infested with adult *Myzus persicae* (Sulz.), carried fewer aphids a week after infestation than did shoots from control tubers. Shoots of tubers from treated plots also grew more slowly than those from the controls.

The aphicidal efficiency of Thimet applied as individual doses separated from the tubers by distances of up to 6 in., decreased as the distance increased, but the effect of distance became less as time passed.

Reasons for the differences in the behaviour of the insecticides are discussed, and the possibilities that the method offers to control virus diseases. The application of insecticides to soil promises to be a useful way of controlling the spread of viruses, provided the harvested crop is free from toxic residues.

Introduction

Insecticides applied as foliar sprays can prevent the spread of potato leaf-roll virus and lessen the spread of virus *Y* (Broadbent, Burt & Heathcote, 1956, 1957) at a cost that is commercially acceptable (Broadbent, Burt & Nix, 1957).

Spraying has disadvantages, however: after the plants have closed across the furrows spraying machinery damages them. Removing diseased plants (roguing) before the crop is vulnerable to damage by machinery can obviate the need for late spraying, but all machinery compacts the soil and this may make extra cultivation necessary. A more serious disadvantage is that aphids are sometimes numerous when the plants are emerging, and it may be difficult to spray early and thoroughly enough to protect the crop during the first 3 weeks after emergence.

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Systemic organo-phosphorus compounds applied to seed or soil have protected some crops from aphids, but little has been published recently about their use with potatoes. Such treatments applied to potato crops might, when combined with roguing, prevent virus spread and avoid the disadvantages of spray treatments. Grainger & Seiby (1949) found that potted potato plants grown in soil treated with parathion at high rates were toxic to aphids (*Macrosiphum solanifolii* (Ashm.)) for 3–8 weeks after planting. Klostermeyer (1953) found that potato plants from cut seed pieces previously soaked in solutions of demeton and schradan were kept free from aphids for 23 days after planting. Demeton was more effective than schradan and its effect persisted longer, but at high concentrations it inhibited plant growth. David & Gardiner (1955) tested schradan, dimefox, sodium fluoroacetate, paraoxon and demeton in a similar way, and found that only plants from tubers soaked in demeton killed *Myzus persicae* (Sulz.) placed on them 23 days after the tubers were treated. Plants grown from tubers treated with 0·1 % demeton killed aphids 63 days after treatment, but this amount must not be exceeded, for 0·2% demeton killed the tubers. Way & Needham (1957) used demeton as a 50% dust with activated charcoal. They placed it in the planting holes with the tubers and, applied in this way at up to 1·0 g. per tuber, it protected the plants from *M. persicae* for 39–112 days without damaging them.

The above observations and other information provided by insecticide manufacturers indicated that these insecticides might harm potato plants, but that the damage was likely to be avoided by placing the insecticide in the soil near the tubers, rather than soaking them in insecticide solution or coating them with solid insecticide before planting. When choosing insecticides for the tests, past performance as a soil aphicide on other crops, probable future availability in suitable formulations, and low mammalian toxicity were the factors mainly considered.

Two experiments are described, designed to test the effect of a single soil application combined with roguing on the spread of potato leaf roll and potato virus *Y*, and to investigate some of the factors affecting the use of insecticides in this way.

Experimental

Control of virus spread (Experiment 1)

Methods

This experiment was similar to previous ones with sprayed insecticides (Broadbent, Burt & Heathcote, 1956, 1957), except that the plots were smaller. This fact, and the layout imposed by the shape of the land into which the experiment had to be fitted (see below), probably made the test particularly severe, and effects on aphids and virus spread were probably less than would be achieved in conditions more typical of potato crops.

A 4 × 4 design was used, with all plots end-to-end in one line but separated from each other by strips of untreated potatoes twenty-two plants wide. Untreated potatoes bordered the line of plots on either side, so that each plot was surrounded by a relatively large area of untreated potato plants. For analysing the results the design was assumed to be a Latin Square. Each plot consisted of five rows of

twenty-five plants containing at its centre one plant infected with leaf-roll virus and one with virus *Y*. The infected potatoes were of the variety King Edward, and the others Majestic. At planting, the furrows were opened and closed by machine, but fertilizer and insecticide were applied and the tubers planted by hand. As both the insecticides used are toxic to man they were applied by people wearing protective clothing, goggles and dust respirators. A granular fertilizer (10 parts N : 10 parts P_2O_5 : 18 parts K_2O) was applied evenly along the furrows at a rate of 10 cwt./acre.

Of the insecticides chosen, one, Thimet, was used as a commercial formulation known as Thimet 44-D which contains: Thimet: active ingredient (*O*,*O*-diethyl *S*-(ethyl-thionomethyl) phosphorothiolothionate), 44%; methylcellulose, 2%; activated charcoal powder, 54%.

The other insecticide, Rogor, was formulated from a technical product containing 90% active ingredient (*O*,*O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolothionate). The formulation contained: technical Rogor, 55·6%; activated charcoal powder, 44·4%.

The following treatments were applied:

Treatment 1. Thimet as a small pile of 44-D containing 0·31 g. active ingredient placed directly beneath each tuber (individual dosing). This rate was about 9·5 lb./acre.

Treatment 2. 44-D mixed with the fertilizer in proportions giving the same rate per acre as in treatment 1.

The 44-D and fertilizer were stirred in a food-mixer and sprayed with enough water to make the mixture sticky. After very thorough mixing, stirring was continued while the mixture was dried with a jet of hot air from a hair-drier until the granules just ceased to cohere; the mixture was completely dried by spreading it on a sheet of paper in a fume-cupboard with a gentle draught. The product, containing 1·9% 44-D, was free-flowing and the insecticide did not separate from the fertilizer when the product was handled.

Treatment 3. Rogor with activated charcoal applied in the same way as treatment 1, at a rate of 0·35 g./tuber or about 10·5 lb./acre active ingredient.

Thimet was applied at a rate which cost a little more than three sprays with DDT miscible liquid at 2 lb./acre, a treatment whose cost combined with roguing was calculated by Broadbent, Burt & Nix (1957). The cost of Rogor was not known when the experiment was planned; it was applied at about the same rate as Thimet.

Treatment 4. An untreated control.

At the chosen dose rates the insecticides were not toxic to potted potato plants when tested in a greenhouse before the experiment.

The crop was planted, with 28 in. between rows and 16 in. between plants in the rows, on 21 April 1958. This spacing is commonly used for ware crops. All the plants had emerged by 6 June, the infected plants were removed on 25 June, the haulm burned off on 15 September, and the tubers lifted on 26 September. For most of the season the weather was cool and wet.

Results

Aphid control. The first alatae were caught in early May (Table 1), but apterae were few until about a week after the crop had completely emerged. The population increased very slowly, reached a maximum about 10 July, declined until 31 July, and remained very small until the crop was lifted (Tables 1 and 2). Potato aphids were few, with *Myzus persicae* the most numerous; the others were mostly *Aphis nasturtii* (Kaltb.) and *Macrosiphum solanifolii*, for *Aulacorthum solani* (Kalt.) were very few (Table 3).

Table 1. *Winged* Myzus persicae *caught in a Moericke water-trap near experimental field*

Period	No. of aphids	Period	No. of aphids
23 May–3 June	2	14–21 July	12
3–10 June	2	21–28 July	11
10–16 June	2	28 July–29 Aug.	0
16–24 June	0	29 Aug.–5 Sept.	1
24 June–1 July	1	5–12 Sept.	3
1–7 July	2	12–19 Sept.	2
7–14 July	14		

Table 2. *Potato aphids/100 leaves throughout season, i.e.* Myzus persicae, Macrosiphum solanifolii, Aphis nasturtii, *and* Aulacorthum solani

	No. of aphids									
Treatment	11 June	24 June	3 July	10 July	18 July	25 July	31 July	11 Aug.	19 Aug.	1 Sept.
Thimet, individual doses (1)	10	4	17	3	48	20	5	22	25	—
Thimet with fertilizer (2)	0	0	0	0	2	2	0	0	0	—
Rogor, individual doses (3)	8	0	0	0	0	0	0	0	0	—
Control (4)	62	148	552	1292	890	368	77	52	38	0

All the treatments greatly decreased the numbers of aphids infesting the plants. Treatment 2 was the most effective and the first to reach aphicidal concentrations in the plants; it kept them almost free from aphids throughout the season. Treatment 3 was almost as effective, although Table 2 suggests that it first appeared in the plants a little later than treatment 2. Although less effective than the other two, treatment 1 checked aphids well at first, but it may have lost efficiency earlier than the others. All treatments were effective long after the infected plants had been removed (25 June).

Three sleeve tests were done (Table 4). The method was that described by Broadbent *et al.* (1956), except that ten aphids per sleeve were left on the plants 3–5 days. A comparison of the aphid control obtained in the sleeve tests done on 11 July (Table 4) with the control of the wild aphid population secured on 10 and 18 July (Table 2) shows that the sleeve tests could predict reliably the potential

performance of the aphicides against the wild aphid population. An earlier test (19 June) showed that treatments 2 and 3 were then fully efficient, but treatment 1 was less efficient than it became later. The test on 13 August, 114 days after planting, showed all treatments to be declining in efficiency, though treatment 2 was still very effective.

Table 3. *Numbers of potato aphids/100 leaves on three occasions*

(Treatments: (1) Thimet, individual doses; (2) Thimet with fertilizer; (3) Rogor, individual doses; (4) Control.)

Date and treatment	*Myzus persicae*			*Aulacorthum solani*			*Macrosiphum solanifolii*			*Aphus nasturtii*		
	Adult			Adult			Adult			Adult		
	Apt.	Alate	Young	Apt.	Alate	Young	Apt.	Alate	Young	Apt.	Alate	Young
24 June												
1	1	1	3	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	22	3	68	2	0	2	3	0	18	8	0	25
10 July												
1	0	0	2	0	0	0	0	0	2	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	85	2	666	0	0	2	13	0	100	75	0	348
25 July												
1	2	0	13	0	0	0	3	0	2	0	0	0
2	0	0	0	0	0	0	0	0	2	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	26	0	97	4	2	22	31	0	183	0	0	0

Table 4. *Sleeve tests on potato plants (Experiment 1)*

(A = ratio of aphids found at end of test to those originally introduced. B = aphids found on treated plants as percentage of those on untreated.)

Treatment	19 June		11 July		13 Aug.	
	A	B	A	B	A	B
Thimet, individual doses (1)	0·96	56	0·58	33	0·83	44
Thimet with fertilizer (2)	0·04	2	0·01	1	0·43	23
Rogor, individual doses (3)	0·18	11	0·38	21	0·77	41
Control (4)	1·70	100	1·78	100	1·89	100

Virus control. At harvest, whole progeny were lifted from five plants in the row on either side of each infected plant and grown in 1959 to estimate virus spread. The results were subjected to an angular transformation and are shown in Table 5 as percentage infection calculated from the transformed values. The few aphids present before roguing spread leaf-roll virus to only a few previously healthy plants (Table 5). Treatments 2 and 3 significantly (at the 5 % level of probability) lessened the spread of leaf roll, but the effectiveness of treatment 1 was doubtful. Control of aphids and of leaf-roll spread were directly related. Only treatment 2 checked the spread of virus *Y* significantly; as in previous experiments using sprays, the spread of leaf-roll virus was decreased more than that of virus *Y*.

Phytotoxicity. No treatment delayed emergence of the plants. At intervals the heights of sixty plants, taken at random from each treatment, were measured (Table 6). Although, when the results were subjected to a log $n+1$ transformation only treatments 3 on 9 June and 2 on 4 July proved significantly different from the control at the 5% level of probability, the consistently low figure for treatment 3 is noteworthy. Treatment 3 also produced lesions on the leaves of young plants.

TABLE 5. *Percentage spread of leaf roll and virus* Y *to the five plants in the row on either side of each infector*

(Detransformed values, see p. 584)

Treatment	Leaf roll	Rugose mosaic (*Y*)
Thimet, individual doses (1)	2·6	19·5
Thimet with fertilizer (2)	0	5·7
Rogor, individual doses (3)	0	18·0
Control (4)	8·1	15·8

TABLE 6. *Effect of treatments on height of plants* (*Experiment* 1)

(Average height of sixty plants (in.).)

Treatment	9 June	18 June	4 July
Thimet, individual doses (1)	3·62	9·47	23·7
Thimet with fertilizer (2)	3·20	9·57	22·1
Rogor, individual doses (3)	2·83	8·05	22·7
Control (4)	3·50	9·52	24·3

The yields from the experiment were as follows: treatment 1, 15·48 tons/acre; treatment 2, 14·95 tons/acre; treatment 3, 14·43 tons/acre; treatment 4, 15·07 tons/acre.

The yields from treated plots did not differ significantly from the control values when the results were subjected to a log $n+1$ transformation, but treatments 2 and 3 gave the lowest values.

Residues in tubers

(1) *Chemical tests*. In mid-November Mr E. I. Johnson of the Government Laboratory kindly analysed chloroform-soluble extracts from the stored tubers for anticholinesterase residues. Treatment 4 did not inhibit cholinesterase and the inhibition by treatment 1 was too slight to measure. Treatment 2 caused inhibition equivalent to 0·2 p.p.m. of oxidized Thimet.

Extracts from tubers given treatment 3 showed anticholinesterase activity equivalent to 5 p.p.m. of Rogor (using Rogor as a standard); Rogor itself is a very weak inhibitor. A chemical method in which phosphorus was determined in the treated chloroform extract showed that neither Rogor nor any other chloroform-soluble, phosphorus-containing residue was present at a greater concentration than 0·1 p.p.m.

(2) *Biological tests*. Way & Needham (1957) found that shoots from tubers directly treated with demeton were toxic to aphids; they found evidence that so also were shoots from daughter tubers set by plants produced by the treated tubers. During the winter of 1958–9, we tested two lots of tubers from Exp. 1 for this effect.

Test 1. On 14 November 1958, thirteen tubers from each treatment, taken at random and therefore of different sizes, were buried 1 in. deep in sterile soil (John Innes no. 1), each in a separate pot; they were stored in a greenhouse at 17–20° C. The shoots emerged over a long period and took from 47 to 84 days to reach a height of 1 in. (Table 8). When they were 1–1½ in. high, the shoots were infested with ten adult apterous *M. persicae*, and a week later the aphids on the shoots were counted (Table 7). Although shoots from treated daughter tubers had fewer aphids than those from untreated daughter tubers, the variation between replicates was too great for the results to be statistically significant.

Table 7. Myzus persicae *found on shoots from daughter tubers* 1 *week after infestation with ten adult apterae*

	Test 1 (13 tubers)		Test 2 (15 tubers)	
Treatment	Average no. of aphids/shoot	% untreated	Average no. of aphids/shoot	% untreated
Thimet, individual doses (1)	56	92	58	95
Thimet with fertilizer (2)	40	66	51	84
Rogor, individual doses (3)	31	51	32	52
Control (4)	61	100	61	100

Table 8. *Average time elapsed between planting daughter tubers for tests* 1 *and* 2 *and growth of their shoots to a height of* 1 in.

	Test 1 (13 tubers)		Test 2 (15 Tubers)			
Treatment	Av. time for growth of shoots to 1 in. (days)	% untreated	Av. time for growth of shoots to 1 in. (days)	% untreated	Av. height at end of test (in.)	% untreated
Thimet, individual doses (1)	66	96	16·9	137	4·4	94
Thimet with fertilizer (2)	64	93	20·3	165	4·6	98
Rogor, individual doses (3)	64	93	19·7	160	2·0	43
Control	69	100	12·3	100	4·7	100

Test 2. This was begun on 3 March 1959. To decrease variability, the soil in the pots was covered with a layer of sand (in which aphids straying from the plants would be less likely to become lost), and the aphids were confined to the plants and the sand surface by lamp-glasses with muslin tops; otherwise the conditions were the same as for test 1. These precautions and the shorter period during which the shoots emerged (which enabled the shoots to be infested more contemporaneously), lessened the variation between replicates, though considerable variation still occurred (Table 7). The time taken for the shoots to reach a height of 1 in. was recorded and, in test 2, their height when the aphids were counted (Table 8).

When the results of both tests were subjected to a log $n+1$ transformation, only in test 2, on shoots from tubers given treatment 3, did the number of aphids on shoots from treated tubers differ significantly (at the 5% probability level) from the number on shoots from untreated tubers. However, taking the two tests together, all insecticidal treatments depressed the aphid populations below the corresponding untreated levels. Treatment 3 consistently decreased the number of aphids to half that found on the control; treatment 2 had less effect and its activity appeared to decline in the period between the tests; treatment 1 had negligible effect. In test 1 shoots from tubers given insecticidal treatments took about the same time to reach a height of 1 in. as shoots from untreated tubers (Table 8), but in test 2 they took from 37 to 60% longer. In test 2 only treatment 3 affected the height reached by the shoots at the end of the test, depressing it to about one-half the untreated value.

Although the initial growth of the shoots was slowed, when potato plants were grown in the field from treated daughter tubers they yielded as much as plants from untreated daughter tubers.

Insecticide placement (*Experiment* 2)

The effect of the distance between dose and tuber on the efficiency of the insecticides when potato tubers were dosed individually was investigated, for this information is necessary before equipment can be designed for placing such a dose mechanically. A micro-plot experiment was done with plots consisting of single rows of ten plants in a 5×5 Latin Square design.

All insecticidal treatments were of Thimet 44-D applied at a rate of 0·31 g. active ingredient per tuber. The five treatments were:

A. Dose placed directly below and in contact with each tuber.
B. Placed at same depth but 3 in. along row from each tuber.
C. Placed 6 in. along row from each tuber.
D. Combined with the fertilizer and applied evenly along row.
E. Control: no insecticide applied.

The planting procedure was as in Exp. 1. The tubers were planted on 21 April and the efficiency of the treatments was assessed on four occasions by means of sleeve tests; the results are shown in Fig. 1.

The first test (3 June) was done when almost all the plants had emerged; no treatment was then fully effective, but treatment D, as in all subsequent tests, was the most effective; even the best treatments (A and D) were not very efficient until about a week after emergence. By 13 June treatment D was giving almost 100% control and it remained highly effective until the last test (17 July). Treatment A, the next most effective, reached its greatest efficiency in test 2 and then declined. Treatments B and C, which were equally effective except in test 1, when B was better than C, did not reach their maximum efficiency until test 4 when they were about as effective as treatment A. It seems likely that the insecticide moved through the soil hardly at all, and that a plant could take it up only when its roots reached the original location of the dose.

As in Exp. 1, the heights of the plants were measured to see whether the insecticide affected growth (Table 9). Although in Exp. 1 Thimet combined with fertilizer (treatment 2) had little or no effect on the plants, in Exp. 2 it lessened their height. This difference may have resulted from the different sizes of the plants in the two experiments. In Exp. 2 the plants in all the plots were smaller and less vigorous than those in Exp. 1; this would have been associated with smaller root systems, remaining longer near the insecticide.

Table 9. *Effect of the location of a dose of Thimet on height of plants (Experiment 2)*

(Average height of forty plants (in.). For description of treatments see text, p. 587.)

Treatment	6 June	18 June	4 July
A	2·36	7·45	21·4
B	—	7·92	21·6
C	—	7·70	21·3
D	2·16	5·90	17·2
E	2·60	7·95	21·5

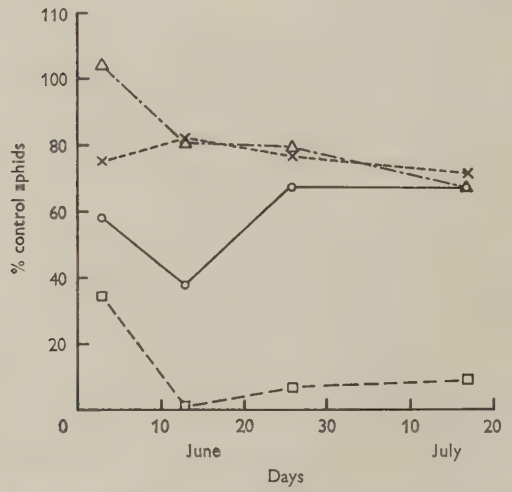

Fig. 1. Effect of the location of a dose of Thimet on its efficiency as an aphicide. Number of aphids found on treated plants in sleeve tests as percentages of number found on controls. Treatments: (A) Individual dose under tuber; (B) dose 3 in. from tuber; (C) dose 6 in. from tuber; (D) applied with fertilizer; (E) control. —○—, Treatment A; – –×– –, treatment B; – –△– –, treatment C; —□—, treatment D.

Discussion

The spread of virus in 1958 was too little to test these control methods rigorously, but the aphid control obtained shows that all treatments, when combined with roguing, should control the spread of leaf roll satisfactorily. It is likely that treatments 3 and 4, at the rates applied, would control spread without roguing or, combined with roguing, could give satisfactory control at lower rates, for aphid

control was good throughout the period when a natural infestation was present and also for some time afterwards.

An interesting conclusion is that, although individual doses of Thimet were not as effective as the same compound combined with the fertilizer, individual doses of Rogor were as efficient as Thimet with fertilizer. The different efficiencies of individual doses of the two compounds may be associated with differences in their water solubilities, rather than with their intrinsic toxicities to aphids. Thimet is rather insoluble in water (50 p.p.m.), whereas Rogor is fairly soluble (2·5%); it seems likely that, as the roots of the plants grew outwards, enough Rogor diffused outward in the soil moisture to keep the plants aphicidal; Thimet was insufficiently soluble, and the absorbing region of the roots soon moved away from the insecticide. On the other hand, Thimet applied with the fertilizer was dispersed initially through a large volume of soil beyond which the roots of the plants were less likely to extend; also, Cooke (1954) showed that the absorptive regions of potato roots tend to proliferate near fertilizer. Systemic compounds not readily soluble in water are probably better applied with the fertilizer, whereas individual doses of water-soluble compounds can be expected to be effective.

Exp. 2 confirmed that Thimet with fertilizer was better than individual doses of Thimet, and also showed that any horizontal separation of the tuber from a single dose of this insecticide lessened its efficiency; this was most evident early in the season. By contrast, the efficiency of single doses of Rogor, for reasons discussed above, is likely to depend less on the distance between dose and tuber.

All treatments were least effective during the week after emergence; apparently the plants took up too little insecticide from any treatment to emerge from the ground fully aphicidal. The reason for this is not known; it may be that the young plants were still drawing nutrients mainly from the seed tuber, or the roots had not reached the insecticides. In seasons when aphids are numerous while the plants emerge, this might prove a serious shortcoming, as much virus is spread then. Temporary protection in these conditions might be provided by pre-emergence soil watering with a systemic insecticide. The depth at which tubers are planted, and, with chitted tubers, the length of the shoot at planting, might also affect the rate of absorption of the insecticide.

Even at the very high rates used, phytotoxicity was slight, and seems unlikely to be troublesome with the materials and formulations chosen.

The chemical analyses suggest that residues in the tubers from treated plants were insufficient in quantity and toxicity to be dangerous to consumers, but the biological tests made later showed that the treated tubers were certainly abnormal. The ability of shoots from these treated tubers to depress aphid populations feeding on them could have resulted from insecticide residues carried into the shoots from the seed tubers, and perhaps concentrated there, or from alterations of the plants' metabolism by the insecticides so that 'treated' shoots were less suitable as food for aphids than 'control' shoots. That the insecticides affected the plants in some way is shown by the decreased height of the 'treated' shoots (Table 8) and this suggests another possibility—that the insecticides may have depressed the aphid

populations simply by lessening the size of the shoots, and, consequently, the amount of food available.

From these experiments it is not possible to decide how the insecticides affected the potato tubers, but it is evident that the tubers were affected. It is interesting that the aphicidal efficiencies of the treatments and, consequently, their capacity for controlling virus spread, are correlated with their degree of phytotoxicity, of yield depression, with the activity of residues left in the tubers, with toxicity to aphids feeding on tuber shoots, and with the extent to which they slow down the growth of these shoots.

The use of soil insecticides for controlling potato virus diseases thus shows promise, but more basic knowledge about the behaviour and subsequent toxicity of such compounds in the soil and their relationship to the plants is needed before the best use can be made of them. Information is particularly needed about the effect of soil conditions and stage of plant growth on their rate of uptake, and about the factors affecting their movement and persistence in soil.

There are two methods of producing seed potatoes: (1) saving small tubers from ware crops, or (2) growing a small area specially for seed. Because residues of the soil insecticides now available are undesirable in tubers harvested for ware, it would be preferable at present to experiment with this means of virus control on crops of which all the tubers will be used as seed (that is, in conjunction with method (2) above). The development of less toxic soil aphicides would make such control methods more acceptable, especially for use on ware crops.

We are grateful to Mr R. de G. Weevers, Mr R. Garrett, and other members of the Rothamsted Staff for their help, and we should also like to thank Mr J. H. A. Dunwoody for the statistical analysis of the results. Messrs Cyanamid Co. of Great Britain kindly provided the Thimet 44-D and Messrs Fisons Pest Control the Rogor.

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THE EFFECTS OF FUNGICIDE-INSECTICIDE SEED TREATMENTS ON EMERGENCE, GROWTH AND YIELD OF IRRIGATED COTTON IN THE SUDAN GEZIRA

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Fungicide-insecticide seed treatment, particularly with methoxyethylmercuriacetate (MEMA) and dieldrin, often improved emergence, growth and yield of irrigated Egyptian-type cotton (*Gossypium barbadense*) in the Gezira area of the Sudan. Such treatment reduced post-emergence wilting and death of seedlings resulting from termite attack, and also reduced pre-emergence mortality probably caused primarily by soil insects, but appeared to have little effect on the incidence of termite damage to tap-roots of mature plants at the end of the season. Termite attack tended to be worse in close rotations, and the beneficial effects of fungicide-insecticide seed treatment were greater in three-course than in four-course rotation plots. In one experiment treatment improved growth and yield of cotton plants in the apparent near-absence of insect pests and diseases, this effect perhaps deriving from control of minor root damage caused by soil insects and/or fungi.

Certain seed treatments, notably those containing MEMA and γ-BHC, were slightly phytotoxic, but at the levels applied—0·043 % mercury/seed and 0·12–0·24 % insecticide/seed—this aspect, although needing further investigation, seems unlikely to be of serious practical significance in the Sudan Gezira where high sowing rates are the rule; any slight phytotoxicity would probably be outweighed by the protective effects of the treatment. Phytotoxicity tended to become more pronounced with increasing number of seeds per sowing hole.

Several hundred thousand acres of irrigated Egyptian-type cotton, *Gossypium barbadense*, are cultivated annually in the Sudan Gezira, the flat plain lying between the Blue Nile and White Nile rivers south of their junction at Khartoum. For many years all the cotton sowing-seed has been treated with Abavit B powder containing about 5·7 % mercury as mercuric chloride-iodide (3 : 1 by weight) or, more recently, with Strong Abavit of about 8·5 % mercury content, both powders applied at 1 : 150 by weight to seed. Treatment is primarily for the control of seed-borne *Xanthomonas malvacearum*, the causal organism of bacterial blight (blackarm, angular leaf spot), which can be responsible for heavy crop losses to Gezira cotton. Recent research (Tarr, 1956) suggests that a short-wet method of seed treatment using the water-soluble organo-mercurial compound methoxyethylmercuriacetate, hereafter referred to as MEMA, is more bactericidal, and application of 50 c.c. of MEMA solution in water per pound of seed to give about 0·043 % mercury/seed or, with very heavily infected seed, 0·086 % mercury/seed was therefore recommended as standard treatment for Gezira sowing-seed.

Fungicide-insecticide seed treatment

In view of the improved stand, growth, and yields resulting from fungicide-insecticide seed treatment of other Gezira crops, notably sorghum (Tarr, 1954), dolichos bean (Tarr, 1958) and wheat (Tarr, 1957), an investigation of this treatment was made with cotton seed and forms the subject of this paper. The X 1730 A variety of *Gossypium barbadense* was used, and trials were in the form of replicated blocks with 5–10 replicates and subplot areas of $\frac{1}{20}$–$\frac{1}{30}$ acre. Statistical analysis of the results was carried out by Mr Paul Roberts, Jealott's Hill Research Station, but is too lengthy to be included here; copies are held at Jealott's Hill and by the present author. Results in the form of percentages were transformed before analysis, using the 'arc sin square root' transformation, and in such cases both transformed and de-transformed means are given, the former in brackets.

Trial in 1955–6. Results of a preliminary experiment in 1955–6 are summarized in Table 1.

Table 1. *Percentage resow, numbers of seedlings per feddan before thinning, and yield of seed cotton in kantars per feddan**

Seed treatment	%/seed Hg	%/seed γ-BHC	% resow	Seedlings/ feddan	Yield k.p.f.*	Greenhouse experiment: % emergence in wet sand Total	Stunted
Abavit powder	0·057	—	2·4	109,400	3·94	93·1 (74·8)	2·0
Abavit powder + BHC	0·057	0·24	2·1	94,900	4·37	86·5 (68·4)	9·8
MEMA short-wet	0·043	—	2·2	104,000	4·52	86·4 (68·4)	5·0
MEMA short-wet + BHC	0·043	0·24	1·9	93,500	4·39	78·0 (62·0)	18·8
Standard error (treatment mean)				±1,400	±0·1	(±2·6)	

* Kantars of seed cotton per feddan: a kantar in the Gezira is 312 lb., and a feddan is 1·038 acres. Hg = mercury, γ-BHC = gamma-isomer of benzene hexachloride. Transformed percentages in parentheses.

Sowing was by hand on 18 August, with resowing on 27 August; percentage resow refers to resown plant holes. Samples of the seed used in the field trial were sown in moist sand in the greenhouse and washed out, examined and counted 14 days later. Emergence was slightly but significantly reduced by MEMA treatment, and highly significantly reduced by insecticide as compared with no insecticide, but not sufficiently so to necessitate above normal resowing, this being small in all treatments. The greenhouse experiment confirmed that the combined dressings were somewhat phytotoxic, and further experiments on this aspect are described later in this paper. No bacterial blight or other above-ground disease was noted and flea-beetle (*Podagrica* spp.) attack was light, although it was observed that seedlings grown from insecticide-treated seed seemed to suffer somewhat less leaf damage than the others. Neither pest nor disease attack on the aerial parts of the plants was sufficient to account for the highly significant yield differences

obtained. No effect on maturity of the crop was apparent. It was thought that MEMA might perhaps have insecticidal properties and that the yield increases might have been associated with 'invisible' damage to the roots by pest and/or disease, this damage being sufficient to result in appreciable crop loss but not sufficient to cause wilting or other obvious symptoms on the aerial parts beyond, perhaps, slight differences in growth.

Trial in 1956–7. In the following season, 1956–7, two plot experiments were carried out, each comprising ten different methods of seed treatment, viz. Strong Abavit powder alone and with two levels (0·12 and 0·24% seed) of γ-BHC and of dieldrin (DN), and MEMA short-wet alone and with the two levels of both insecticides. MEMA + insecticide mixtures were applied in one operation using 50 c.c./lb. of seed, but with Abavit + insecticide treatments the Abavit powder was first thoroughly mixed with the seed and the insecticide was then applied as a short-wet treatment at 50 c.c./lb. The seed was sown in a three-course rotation plot (*cotton*–fallow–sorghum) on 19 August (Table 2), and in an eight-course plot (cotton–fallow–sorghum–dolichos–fallow–*cotton*–fallow–fallow, i.e. cotton twice in 8 years) on 20 August (Table 3). Both plots had received ammonium sulphate fertilizer equivalent to 80 lb. nitrogen/acre. Resowing was 2 weeks after sowing and, as standard practice at the Gezira Research Farm where this work was done, γ-BHC was ploughed into the soil to minimize termite damage when thinning and re-ridging were carried out at the end of September. Observations on percentage resowing, percentage dead plants just before thinning, plant height, growth and yield were recorded. After the final picking in early April 1957 the cotton plants were pulled out, and total numbers of plants and of those with tap-roots showing termite injury were recorded; such plants had short severed tap-roots whose function had often been assumed by one or more lateral roots, as described by Crowther & Barlow (1943). Some of the extensive data are summarized in Tables 2 and 3.

In the three-course experiment (Table 2) seed treatment with MEMA resulted in highly significantly less resowing than with Abavit. During September wilting and death of plants were observed in all treatments, eventually amounting to 11·6% in plants from Abavit-treated seed; it was reduced to nearly negligible proportions by insecticidal seed treatment, particularly with dieldrin. Similarly, plant populations at the end of the season were highly significantly greater where insecticide-treated seed had been sown. Examination of wilting and dead plants suggested that termite attack of the tap-root just below soil level was responsible, the hollowed-out cavities caused by this insect being present and also, frequently, the insects themselves. Dead plants were brown and withered, but tended to remain upright in the soil. Often, but not invariably, all plants in an affected plant hole were killed, and wilting tended to occur in patches rather than scattered at random. The standard practice of ploughing in γ-BHC at the time of thinning and re-ridging (end of September) was followed, but this would not affect termite attack on young plants less than 4–5 weeks old, although it should give some protection to older plants. In fact there were no significant differences in the percentages of termite-injured plants in the

TABLE 2. *Results on three-course rotation plot*

Seed treatment	%/seed Hg	%/seed γ-BHC or DN	% resow	% dead plants	Mean plant height (cm.) 19·9	18·10	19·11	No. of plants/ feddan	% termite injured	Yield k.p.f.
Abavit	0·057	—	10·9 (19·3)	11·6	22·3	41·2	72·9	22,100	22·4 (28·2)	9·49
Abavit+BHC	0·057	0·12	7·4 (15·8)	2·1	23·7	45·4	75·4	23,200	19·5 (26·2)	10·57
Abavit+BHC	0·057	0·24	7·7 (16·1)	0·7	23·2	44·1	74·9	23,500	22·2 (28·1)	10·72
Abavit+DN	0·057	0·12	8·6 (17·0)	0·1	22·9	44·9	75·7	24,200	21·9 (27·9)	10·64
Abavit+DN	0·057	0·24	7·9 (16·4)	0·1	23·6	47·6	79·0	23,600	21·7 (27·8)	11·07
MEMA	0·043	—	4·3 (11·9)	8·5	23·4	46·0	78·3	22,700	21·6 (27·7)	10·50
MEMA+BHC	0·043	0·12	4·2 (11·9)	1·9	23·3	45·9	77·3	24,200	20·7 (27·1)	10·75
MEMA+BHC	0·043	0·24	8·3 (16·8)	0·1	22·6	45·1	76·9	23,600	29·0 (32·6)	10·58
MEMA+DN	0·043	0·12	5·8 (14·0)	0·3	23·2	45·1	76·5	25,000	17·4 (24·7)	11·09
MEMA+DN	0·043	0·24	4·9 (12·8)	0·1	24·7	48·7	80·5	24,400	18·9 (25·8)	10·72
Standard error (treatment mean):			(±1·6)	—	±0·4	±1·2	±1·3	±500	(±2·2)	±0·24

Transformed percentages in parentheses.

TABLE 3. *Results on eight-course rotation plot*

Seed treatment	%/seed Hg	%/seed γ-BHC or DN	% resow	% dead plants	Mean plant height (cm.) 19·9	22·10	15·11	No. of plants/ feddan	% termite injured	Yield k.p.f.
Abavit	0·057	—	6·1 (14·3)	6·3	23·2	48·0	77·3	23,900	9·0 (17·5)	10·68
Abavit+BHC	0·057	0·12	4·4 (12·1)	0·4	24·2	50·0	79·1	24,600	9·0 (17·4)	11·05
Abavit+BHC	0·057	0·24	3·6 (10·9)	0·2	23·4	48·3	77·3	25,000	10·2 (18·7)	11·25
Abavit+DN	0·057	0·12	3·6 (10·9)	0·0	24·9	51·4	79·8	25,100	7·6 (16·0)	11·11
Abavit+DN	0·057	0·24	2·4 (9·0)	0·0	25·2	55·4	85·6	25,000	5·6 (13·7)	11·50
MEMA	0·043	—	2·1 (8·4)	0·6	23·6	48·4	76·9	24,700	7·7 (16·1)	10·75
MEMA+BHC	0·043	0·12	3·0 (10·0)	0·1	23·6	49·8	79·5	23,700	10·4 (18·8)	11·31
MEMA+BHC	0·043	0·24	3·3 (10·5)	0·1	22·6	47·8	78·0	24,200	12·4 (20·6)	11·27
MEMA+DN	0·043	0·12	2·6 (9·4)	0·1	24·7	52·5	82·8	24,200	8·2 (16·6)	11·41
MEMA+DN	0·043	0·24	2·9 (9·8)	0·1	23·7	48·0	77·8	24,200	7·8 (16·2)	11·10
Standard error (treatment mean);			(±1·2)	—	±0·4	±1·3	±1·6	±500	(±0·8)	±0·20

Transformed percentages in parentheses.

treatments at the end of the season, although the two MEMA + dieldrin treatments appeared to be somewhat less affected than the others. Plants grown from Abavit-treated seed grew less well than those from the other nine treatments, as judged by height measurements and visual estimates of growth; slight but consistent differences were evident soon after sowing and were thereafter maintained fairly consistently. In general, MEMA was more beneficial than Abavit, and MEMA + insecticide than Abavit + insecticide, although the last-named was better than Abavit alone. The higher-level dieldrin treatments appeared to be the most effective. Yield from Abavit-treated seed was about a kantar less than that from MEMA-treated seed, and 1·1–1·6 kantars less than that from seed treated with fungicide + insecticide, with dieldrin perhaps slightly (not significantly at $P = 0{\cdot}05$) more effective than the corresponding γ-BHC treatment. There were no marked differences in maturity of the crop, except that the higher-yielding treatments tended to be very slightly earlier.

There was very little above-ground disease or pest attack, apart from a small amount of bacterial blight which was not sufficient to account for yield and growth differences of the order obtained. It seemed possible that seed treatment with MEMA + insecticide, Abavit + insecticide or MEMA alone, in that order, but not Abavit alone, had given protection against a soil-pest complex with termites predominating (or pest-disease complex) attacking the roots of the young plants, and had thereby resulted in improved emergence, growth and yields.

The second experiment was sown in an eight-course rotation plot, with cotton grown twice in 8 years as opposed to once in 3 years in the previous experiment (Table 3). Resowing and plant mortality, the latter due apparently to termites as described above, were considerably less in this experiment, and both were reduced by seed treatment with MEMA or fungicide + insecticide as compared with Abavit alone. As in the previous experiment, but to a lesser extent, plant growth was improved by several of the fungicide + insecticide seed treatments but not by MEMA alone. It is possible that the two 0·24% γ-BHC treatments and the 0·24% dieldrin + MEMA treatment were slightly phytotoxic in both experiments, but that under the conditions of severer termite attack in the first experiment the greater insecticidal power of these three treatments more than compensated for their slight phytotoxicity. Insecticidal seed treatment resulted in highly significant yield increases as compared with purely fungicidal treatment. No marked effect on maturity was apparent. Percentages of plants with termite-injured tap-roots at the end of the season were less than half those recorded in the three-course rotation experiment (Table 2), and tended to be somewhat lower with MEMA- or dieldrin-treated seed as compared with Abavit, Abavit + BHC and MEMA + BHC; in this respect dieldrin, particularly at the higher application rate, was highly significantly better than γ-BHC.

Phytotoxicity of MEMA-insecticide seed treatment

In the experiments described above there were indications that certain fungicide-insecticide seed treatments, notably MEMA + BHC, were slightly phytotoxic, although not of much practical importance at the mercury and insecticide levels used. Further investigation of this was carried out and the results of three field trials are summarized in Tables 4–6.

Table 4. *First phytotoxicity trial: percentage total emergence and percentage normal seedlings*

% γ-BHC/seed	No mercury: % emergence		0·043 % mercury/seed: % emergence		0·086 % mercury/seed: % emergence	
	Total	Normal	Total	Normal	Total	Normal
0·00	82·4 (65·2)	68·9 (56·1)	82·3 (65·1)	73·3 (58·9)	74·5 (59·7)	62·2 (52·1)
0·06	—	—	71·9 (58·0)	63·5 (52·8)	78·4 (62·3)	67·9 (55·5)
0·12	—	—	74·0 (59·4)	66·2 (54·4)	67·0 (54·9)	52·9 (46·6)
0·24	—	—	71·7 (57·8)	54·6 (47·6)	63·4 (52·8)	48·2 (44·0)
0·36	—	—	68·8 (56·0)	47·9 (43·8)	60·2 (50·9)	38·4 (38·3)

Standard error (treatment mean):
% total emergence (transformed): (±2·1)
% normal emergence (transformed): (±2·0)
Transformed percentages in parentheses.

Table 5. *Second phytotoxicity trial: percentage total emergence and percentage normal seedlings*

% insecticide/seed	No insecticide: % emergence		γ-BHC: % emergence		Dieldrin: % emergence	
	Total	Normal	Total	Normal	Total	Normal
0·00	87·8 (69·6)	85·4 (67·5)	—	—	—	—
0·12	—	—	85·8 (67·9)	79·3 (63·0)	82·8 (65·5)	78·1 (62·1)
0·24	—	—	87·0 (68·8)	79·1 (62·8)	82·4 (65·2)	75·6 (60·4)
0·36	—	—	85·6 (67·7)	72·0 (58·0)	85·4 (67·6)	79·6 (63·1)
0·48	—	—	84·4 (66·7)	70·2 (56·9)	82·8 (65·5)	77·0 (61·4)

Standard error (treatment mean):
% total emergence (transformed): (±1·1)
% normal emergence (transformed): (±1·1)
Transformed percentages in parentheses.

In the first trial (Table 4) the MEMA and MEMA-BHC treatments were all short-wet, using 50 c.c. per lb. of seed. 'Normal' seedlings excluded those of below average growth and those plainly stunted or injured when the seedlings were examined and counted 3 weeks after sowing. Phytotoxicity symptoms included general stunting, failure of the cotyledons to unfold, and short, thickened primary roots often brownish in colour, without branches and evidently arrested in growth soon after germination had begun. Toxicity tended to be more pronounced at the higher mercury level and at the higher γ-BHC levels; 0·24 and 0·36% γ-BHC/seed were appreciably toxic at the lower mercury level, and 0·12, 0·24 and 0·36% at the higher level.

A second trial with MEMA-BHC and MEMA-dieldrin treatments was sown in

cooler weather than the previous one, and phytotoxicity was less pronounced (Table 5). All treatments were short-wet at 50 c.c./lb. of seed and 0·043 % MEMA/seed. Dieldrin treatments slightly reduced total emergence as compared with no insecticide, and percentage normal seedlings was somewhat less in all the MEMA-insecticide treatments as compared with MEMA alone (particularly in the two highest γ-BHC treatments).

Other experiments indicated that phytotoxicity of MEMA-BHC seed treatment became more pronounced with increasing number of seeds per sowing hole. In the Gezira this number may range from less than ten to more than thirty, and results of a trial with three seed rates and three γ-BHC levels are summarized in Table 6. All were short-wet treatments at 50 c.c./lb. of seed, with 0·043 % mercury/seed as MEMA.

TABLE 6. *Trial of different seed rates and BHC levels: percentage total emergence and percentage normal seedlings*

% γ-BHC /seed	10 seeds/hole: % emergence		20 seeds/hole: % emergence		30 seeds/hole: % emergence	
	Total	Normal	Total	Normal	Total	Normal
0·00	79·0 (62·7)	69·9 (56·8)	67·1 (55·0)	54·4 (47·5)	62·5 (52·2)	49·7 (44·8)
0·12	65·1 (53·8)	55·6 (48·2)	55·6 (48·2)	44·8 (42·0)	47·8 (43·8)	36·2 (37·0)
0·24	63·2 (52·7)	50·9 (45·5)	58·4 (49·8)	40·2 (39·4)	40·8 (39·7)	25·7 (30·5)
0·36	62·7 (52·4)	52·1 (46·2)	46·4 (43·0)	33·2 (35·2)	42·0 (40·4)	26·9 (31·2)

Standard error (treatment mean):
% total emergence (transformed): (± 1·8)
% normal emergence (transformed): (± 1·5)
Transformed percentages in parentheses.

Phytotoxicity, as judged by emergence and number of abnormal seedlings, increased with increasing number of seeds per hole. This was so even taking into account the reduced emergence due to increased competition and overcrowding inevitably resulting from numerous seeds in a hole.

DISCUSSION

Assuming that in both the experiments recorded in Tables 2 and 3 the results are to be ascribed mainly to control or otherwise of root damage to the young cotton plant by the same soil pest(s) (probably mainly termites) or pest-disease complex, the damage was clearly more severe in the three-course rotation plot. Termite attack tends to be worse in closer rotations (Crowther & Barlow, 1943), and further investigation may confirm the greater effectiveness of fungicide + insecticide seed treatment in such rotations; in this connexion it is interesting to note that, for reasons of land utilization, future expansions of the Gezira irrigated area are likely to be three-course.

Termites are not usually regarded as a major factor influencing Gezira cotton yields, but this view is perhaps based largely on the observed incidence of adult plants killed by this pest. The possibility that they (or other soil insects, including chafer grubs), either alone or as a pest-disease complex, may cause widespread

root damage sufficient to reduce yields but not usually sufficient to cause death, or other conspicuous above-ground symptoms, merits investigation. Thus termite lesions, not sufficiently severe to cause death or severance of the root, were occasionally observed on tap-roots and thicker lateral roots of cotton plants in the Gezira, and presumably resulted from superficial nibbling by termites. Exceptionally, plants were found in which these insects had eaten into and up through the core of the root without destroying the plant (Crowther & Barlow, 1943). It is possible that soil-inhabiting fungi invade the roots through such termite lesions, thereby adversely affecting growth and yield. Consequently, the apparent reduction in termite damage following seed treatment with MEMA might perhaps result from better control of these root-invading fungi rather than from direct effect of MEMA on termites. If, as seems to be the case, the beneficial action of combined fungicide-insecticide seed dressings described above was associated with control of termite damage, this may be divided into three types:

(1) Pre-emergence mortality, in which the germinating seed is destroyed before emergence above soil level. This aspect is reflected in the lighter resowing where insecticide-treated seed was used in the experiments described above. It is important to note that there is no direct evidence that termites were in fact responsible for this pre-emergence mortality, and other insects may well have been implicated. The likelihood that insects rather than fungi were the primary cause is suggested by the much better control given by fungicide-insecticide seed dressings as compared with purely fungicidal ones, especially in the Abavit series (as discussed above it may be that MEMA itself has weak insecticidal or insect-repelling properties).

(2) Post-emergence mortality, in which termites attack and sometimes sever the tap-root of the young plant, thus causing wilting and death. This occurred in September during the interval between emergence and thinning, and was effectively controlled by insecticidal seed dressings and, to a lesser extent, by MEMA alone. Abavit alone seemed to be relatively ineffective in this respect, but no untreated control (i.e. untreated seed) was possible in these experiments.

(3) Post-emergence injury to the tap-root, which thereafter ceased to elongate, its function being taken over by one or more lateral roots without death of the plant; such plants showed short severed tap-roots at the end of the season. According to Crowther & Barlow (1943) this type of damage usually occurs during the first 2 months after sowing and its incidence is positively correlated with that of plants killed by termites. In the experiments described above the extent of this damage was clearly less in the eight-course than in the three-course rotation plot, but only with dieldrin was there any indication that seed treatment had reduced it, and even so only to a relatively slight and uncertain extent. It may be that this type of termite damage occurs when the insecticide in the seed dressing has been dissipated, possibly 5–6 weeks after sowing with γ-BHC and somewhat longer with dieldrin, a more persistent insecticide. It is interesting to note that fungicide-insecticide seed dressings have recently been reported to improve stand of rain-grown American cotton (*Gossypium hirsutum*) in the central Sudan (Clinton, 1959);

the pathogens or insects responsible for pre-emergence mortality were not known, but most of the post-emergence losses were due to termites and millipedes.

It has been suggested by Crowther & Barlow (1943) that the shed seed coats of germinating cotton seedlings attract termites and, if this is so, it is reasonable to suppose that the insecticidal seed dressings used in the experiments described in the present paper rendered the discarded seed coats repellent or destructive to termites and thereby protected the nearby seedlings. As the insecticide gradually disappeared so would the chances of termite attack increase, but by this time the plants would be larger and perhaps better able to tolerate attack.

The possibility that termites might be secondary invaders rather than the primary cause of wilting should be borne in mind. Thus Pollard (1956) reported that, with irrigated dolichos bean and groundnut in the Sudan Gezira, termites show a strong preference for dead plants and may perhaps be secondary invaders which complete the destruction of tissue damaged by other agents, e.g. cockchafer grubs, *Schizonycha* sp. The latter grub causes wilt diseases of sorghum, dolichos bean, and other plants in the Gezira (Tarr, 1954, 1958) and there are occasional reports of grubs attacking cotton (e.g. Cowland, 1933). Dr Pollard (personal communication) found chafer grubs associated with roots of about 25% of the living cotton plants he examined at the Gezira Research Farm, where the present work was carried out, although no obvious root damage was seen. The possibility that termites may be secondary invaders of grub-damaged cotton plants needs investigation; as pointed out by Dr Pollard, termites are widespread in Gezira soil yet termite damage to cotton plants tends to be very localized, as is the distribution of chafer grubs. On the other hand, Crowther & Barlow (1943) list examples of damage by termites to living plants, and in the experiments described in the present paper there was no positive evidence that other insects were involved; further investigation is needed, including study of the soil insects and micro-organisms associated with cotton roots in the Gezira.

The slightly phytotoxicity of MEMA-insecticide seed dressings, at the levels used in the present investigation, seems unlikely to be of practical significance in the Gezira, and would probably be outweighed by the beneficial effects resulting from protection of the seed and young seedlings from harmful soil pests and fungi. Cotton is sown in spaced holes along the irrigation ridges and a high seed rate (often exceeding 100,000 seeds per acre) is largely unavoidable, in that about ten or more emerging seedlings per plant hole are often needed to break through the hard, dry crust which not infrequently forms after irrigation and subsequent drying of the heavy clay surface soil. The plants are later thinned to about three per hole, more young plants being discarded than are kept. From these considerations it is apparent that the slight phytotoxicity shown by some MEMA-insecticide seed dressings, at the rates used in this investigation, is not likely to necessitate higher seed rates or increased resowing, but further investigation of this aspect is desirable.

The promising results described in this paper suggest that fungicide-insecticide treatment of cotton seed is likely to prove a simple and inexpensive method of improving crop establishment and early growth of cotton in the Gezira and probably

elsewhere in the Sudan (and, indeed, in other countries where the crop is cultivated). The use of systemic insecticides in cotton seed dressings has given promising results in the United States and Brazil, and merits investigation in the Sudan; seed treatment as a crop protection measure for cotton has recently been reviewed (Tarr, 1959).

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EXPERIMENTS ON THE HERBICIDAL ACTION OF 1,1′-ETHYLENE-2,2′-DIPYRIDYLIUM DIBROMIDE

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(With 6 Text-figures)

Light was shown to increase the rate of kill obtained with 1,1′-ethylene-2,2′-dipyridylium dibromide, without being essential for herbicidal action. The light effect, which was observed only in green tissues, increased with intensity up to about 10 k-lux, and was inhibited by *N*-(4-chlorophenyl)-*N*,*N*′-dimethyl urea or the absence of oxygen, but not by potassium cyanide or in a carbon-dioxide-free atmosphere. The herbicide was also shown to stimulate and inhibit respiration. The stimulation was transitory, and appeared to depend on a continuous supply to the tissues, while the inhibition did not.

These observations are interpreted, consistently with chemical evidence, to show that the reduction of the herbicide to its free radical form is an essential step in the sequence of toxic reactions. It is supposed that energy for reduction comes from the photolysis of water in the light, and from respiration in the dark. Once formed, the radical may initiate chain reactions in the presence of oxygen which end in the degradation of essential cytoplasmic components.

Introduction

1,1′-Ethylene-2,2′-dipyridylium dibromide (diquat) is a rapidly-acting herbicide causing wilt and desiccation but none of the abnormal growth responses associated with hormone-type herbicides. Under suitable conditions it is translocated within the plant, but usually gives a top kill only (Brian, Homer, Stubbs & Jones, 1958; Stubbs, 1958). It is one of a series of quaternized dipyridylium compounds, many of which are herbicides of a similar kind.

Homer, Mees & Tomlinson (1960) and Homer & Tomlinson (1959) showed that those compounds of the series having the same herbicidal action as diquat could be reduced to a free radical form by the addition of an electron, and conversely that compounds which could not be so reduced were not highly herbicidal. With certain plants the degree of herbicidal activity was related to the oxidation-reduction potential of the substances under test; those that were the more easily reduced were the more powerful herbicides. This suggested that the herbicides must be reduced to the free radical form within the plant before they can kill it. The experiments described here show how the reduction may be effected and also provide some indication of what further reactions may follow radical formation.

Methods

Broad beans, *Vicia faba* (var. Sutton's Prolific Longpod), were used in most experiments. The crop plants were raised in a glasshouse and weed species were brought in from the field.

The effects of treatments given in the light and dark were often compared. Light referred to without qualification is daylight, the darkened material usually being kept in a box or under glazed jars adjacent to the light treatments in the glasshouse, to minimize differences in temperature. Some experiments were done in controlled-environment rooms where the temperature in light and dark was the same, although the humidity may have varied. The light came from nine mercury vapour and two neon lamps giving an intensity of 20 k-lux at the level of the plants.

Herbicidal activity was assessed in three ways:

(1) By observation of symptoms in treated material. The method of treatment varied; large plants such as broad beans had one leaflet dipped into a tube holding 15 ml. of an aqueous solution of diquat, while smaller plants were treated through the roots or by dipping the basal end of the cut stem into a solution. Single leaves were also used when the habit of the plant (e.g. clover) made this more convenient, the petiole then being placed in a solution of the herbicide.

(2) By measuring the rate of blackening of small discs, 3·5 mm. in diameter, cut from broad-bean leaves. One large leaflet furnished up to 120 discs. When floated on solutions of the herbicide in the light, but not in the dark, the discs turned black, the colour spreading inwards from the edge, so that the number of completely black discs could easily be counted at intervals. Old leaves gave a more satisfactory colour than young ones, and it was important that the discs should remain floating and not sink into the treating solutions. The rate of blackening varied greatly between discs cut from leaves in the same position on different plants, or from different positions on the same plant. All the leaflets of one leaf, however, behaved sufficiently alike to be used when a large number of discs were needed. Twenty to thirty, cut at random, were used for each treatment.

(3) By measurement of the effect on oxygen consumption and carbon dioxide evolution. Discs of 8·5 mm. diameter were cut from broad-bean or mustard (*Brassica alba*) leaves and floated on solutions of the herbicide. Each solution received one disc from every leaf used. There were two or three replicates per treatment, each of fifteen discs. 1 cm. pieces from the tips of broad-bean roots were also used in a few experiments.

After treatment the plant material was washed, dried with filter-paper, and put into Warburg manometer flasks on 2 ml. of M/50 tris-hydroxymethylamino-methane—sulphuric acid buffer, pH 7·0. Oxygen uptake was measured in the usual way, and carbon dioxide evolution by the method of similar treatments in adjacent flasks, one with and one without alkali in the centre well. The measurements of gaseous exchange were made after treatment with the herbicide, because it was frequently necessary to compare the effect of diquat in the light and dark, and the Warburg apparatus was not equipped for this. The flasks were kept in the dark

between readings of the manometers made by normal electric light. The first readings were taken 60–150 min. after the plant material had been removed from the herbicide solutions, when the various manipulations and the attainment of temperature equilibrium were completed.

The rate of oxygen consumption of both control and treated material varied during the period of measurement, but for some purposes these variations have been ignored, and the mean rate of oxygen consumption over a 2 hr. period computed for each treatment. It was found that in the relevant experiments the changes during the period of measurement were small compared with the changes during the period of treatment.

Preliminary experiments showed that the variation in oxygen uptake between individual replicates of the same treatment might be as high as 20% for broad-bean leaf discs but between 5 and 10% for mustard leaf discs.

Experimental

The effect of light on herbicidal activity

Broad-bean plants were treated for 24 hr. in the dark with 3×10^{-3}M diquat; the leaflet through which the treatment had been given was then cut off so that no more of the herbicide could enter. Half the plants left in the dark remained normal in appearance for the next few hours. The other half were put in the light, and within 10 min. the leaves adjacent to the treated one had begun to blacken; after about 2 hr. they began to wilt. Therefore, the herbicide had been taken up during treatment in the dark, but had no visible toxic action until the plants were put in the light.

Experiments in which the treated leaflets were not removed from the plants showed that the herbicide could eventually kill broad beans in the dark. Plants treated in this way began to wilt after 48–72 hr., and were dead in 5 days, while controls survived unharmed. It was therefore concluded that light speeded up the rate of kill, but that it was not essential for herbicidal action. There was an obvious difference in the order of appearance of toxic symptoms in light and dark; in the light blackening preceded wilting, but in the dark the black colour appeared only in very wilted plants.

Experiments in controlled-environment rooms confirmed that, as with broad beans, light hastened the response to the herbicide of potato (*Solanum tuberosum*) var. Great Scot, black nightshade (*S. nigrum*), field madder (*Sherardia arvensis*), broad red clover (*Trifolium pratense*), petty spurge (*Euphorbia peplus*), and wheat (*Triticum vulgare*) var. Cappelle. The magnitude of the light effect varied with the species, and perhaps also with the growing conditions. For example, potatoes raised in June showed a large light-response, but those raised in September showed only a small one. None of these species showed the clear difference in the type of response to the herbicide in light and dark found with broad beans, and none developed a black pigment.

The effect of different light intensities on the rate of blackening of broad-bean

leaf discs was also investigated. Discs were put in a controlled-environment room in Petri dishes whose sides and base had been made light-proof. The intensity of the incident light was varied by screening the lids with layers of muslin. Fig. 1*a* shows the time course of blackening in one experiment on discs treated with a 5×10^{-4}M solution of the herbicide in the dark for $3\frac{1}{2}$ hr. before being put in the light at 26° C. In Fig. 1*b* the time taken for half the discs to blacken is plotted against light intensity, and it will be seen that the maximum rate was reached with an intensity in the region of 10 to 12 k-lux. A similar value was obtained in four other experiments, but seven more gave maxima in the region of 5–8 k-lux.

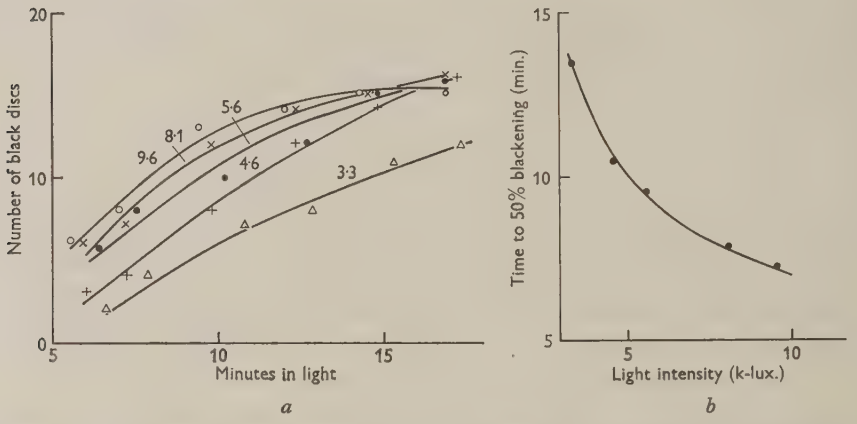


Fig. 1 *a*, *b*. The effect of light intensity on rate of blackening of bean discs. Details of the treatment are given in the text. *a* shows the rate of blackening at five light intensities given (in k-lux) by the figures on the curves; *b* shows the time taken for 50 % of the discs (8) to blacken at each intensity.

A precise figure for the saturating light intensity is less important than the demonstration that the input of energy needed to drive at least this particular reaction to the herbicide was high. The production of the black pigment in broad beans, although a characteristic response to diquat, was apparently not essential to the death of the plant. It could be inhibited by potassium cyanide in concentrations which did not prevent wilting and a depression of oxygen uptake in treated plants.

The light effect and chlorophyll

Manometric experiments showed that isolated broad-bean roots were not more rapidly effected by diquat in the light than in the dark. An experiment was then done on green and etiolated wheat seedlings (var. Capelle) to find whether the light effect in leaves depended on the presence of chlorophyll. The etiolated material was grown entirely in the dark, and all the plants were treated in dim light by dipping the leaves for 15 sec. in a 10^{-2}M solution of the herbicide. Some green and some etiolated plants were then put in the light or dark in controlled-environ-

ment rooms at 26° C. Green wheat in the light began to show scorch after 24 hr. and was almost dead after 4 days. In the dark, loss of turgor was noticeable 40 hr. after treatment, and at the end of 4 days the plants were very wilted but not dead. Etiolated plants in both environments behaved like green plants in the dark, the first signs of wilting appearing after 40 hr. The experiment was greatly facilitated by the fact that the etiolated plants which had been dipped in the herbicide and put in the light failed to develop a green colour; without chlorophyll they responded as though they had been left in the dark. Also, the growth of both green and etiolated wheat was greatly retarded from about the time when the first signs of wilting were noticed.

Experiments with inhibitors of photosynthesis

The requirement for chlorophyll, and the high light intensities needed to give the maximum rate of pigment production in broad-bean leaves, suggested that a photosynthetic reaction might be involved in the response to diquat in the light. To test this, a study was made of the interaction between the herbicide and *N*(4-chlorophenyl)-*N*,*N*′-dimethyl urea (monuron), a powerful inhibitor of the Hill reaction in preparations of isolated chloroplasts (Wessels & van der Veen, 1956; Spikes, 1956).

Single leaflets of intact broad-bean plants were dipped for 18 hr. in 10^{-4}M monuron, and then put in a mixture of 10^{-4}M monuron plus 10^{-3}M diquat. In comparison with plants treated with diquat alone, this treatment delayed death in the light by about 24 hr., but had no effect on the response of plants in the dark. Orache (*Atriplex patula*), mustard (*Brassica alba*), groundsel (*Senecio vulgaris*), field madder (*Sherardia arvensis*), petty spurge (*Euphorbia peplus*), plantain (*Plantago major*), black nightshade (*Solanum nigrum*), sugar beet (*Beta maritima*), kale (*Brassica oleracea*), and broad red clover (*Trifolium pratense*), also responded to treatments with monuron and the new herbicide in the same way, although the delay in onset of death was usually smaller than for the broad bean. Among the species tested only linseed (*Linum usitatissimum*) failed to show any inhibition of the activity of diquat when treated with monuron. Further experiments with linseed showed also that the speed of action of the herbicide was the same in light and dark.

The rate of blackening of broad-bean leaf discs also was inhibited by monuron treatment applied either before or after treatment with diquat. The action of monuron cannot, therefore, be attributed to inhibition of uptake of the herbicide. The degree of interaction between the two compounds varied with leaves from different plants, but a very low concentration of monuron was sometimes sufficient to cause inhibition (Fig. 2). A complete inhibition of activity could be shown with 10^{-5}M monuron. Thus after an 18 hr. pretreatment, broad-bean leaf discs were put for $2\frac{1}{2}$ hr. in the light on a mixture of 10^{-5}M monuron with 10^{-4}M of the new herbicide. The mean oxygen uptake over a subsequent 2 hr. period was 43 μl./15 discs/hr., compared with 45 μl./15 discs/hr. for discs treated with monuron alone, and 22 μl./15 discs/hr. for discs treated with diquat alone.

Potassium cyanide, in doses which depressed respiration, failed to inhibit the effect of the herbicide on oxygen uptake. Indeed cyanide treatment sometimes appeared to enhance herbicidal activity: for example, mustard leaf discs were left 18 hr. in the dark on a 10^{-3}M solution of diquat, 10^{-3}M cyanide was then added, and the discs left on the mixture for another 2 hr. in the dark, then put in the light for 100 min. The mean oxygen uptake in a 2 hr. period was 1 μl./15 discs/hr., compared with 55 μl./15 discs/hr. for a treatment with diquat alone, and 97 μl./15 discs/hr. for the treatment with cyanide alone. In this experiment cyanide alone had no effect on oxygen uptake, the figure for a water control being 96 μl./15 discs/hr. Cyanide also enhanced herbicidal activity measured by manometric experiments with bean leaf discs, although at the same time it inhibited the formation of the black pigment.

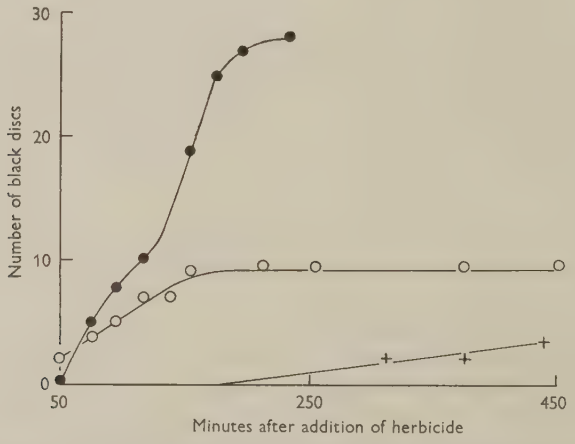


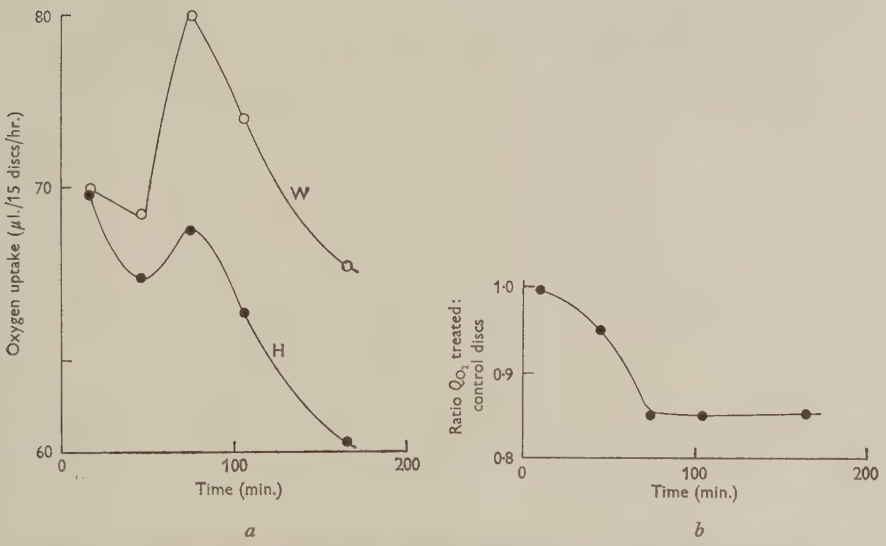
Fig. 2. The effect of monuron on the rate of blackening of bean discs. There were twenty-eight discs in each treatment. ●—●, Pretreated with water; ○—○, pretreated with 5×10^{-7}M monuron; +—+, pretreated with 5×10^{-6}M monuron. All discs then treated with 10^{-3}M 1,1′-ethylene-2,2′-dipyridylium dibromide.

The effect of cyanide on the photosynthesis of the leaves was not measured, but it is known to inhibit carbon dioxide uptake, rather than the earlier stages of photosynthesis. The results therefore suggested that the increased rate of kill with the herbicide should be obtained in the light in the absence of carbon dioxide. To confirm this, broad-bean leaf discs were exposed to the herbicide in the dark in a stream of carbon-dioxide-free air for several hours. When put in the light they blackened at the same rapid rate as discs similarly treated in a stream of normal air.

The effect of the herbicide on respiration

The rate of oxygen consumption in the manometric experiments increased with time to a maximum, sometimes with an inflexion or dip in the rising arm of the curve (Fig. 3*a*). The shapes of the curves for treated and control discs were

generally similar so only the relationship between the rates of oxygen consumption of the two was studied. This ratio declined from an initial high value to a constant figure during the period in which measurements were made (Fig. 3*b*). A similar change with variations in the initial and final constant values of the ratio was always found, except when the oxygen consumption had been depressed to a very low value by the herbicide treatment.



a *b*

Fig. 3*a*, *b*. Effect of the herbicide on oxygen consumption of bean discs. The discs were treated for 3 hr. in the light with water (W) or 10^{-3} M herbicide (H) before the measurements were made. The first readings were taken 105 min. after the discs had been taken off the treating solutions.

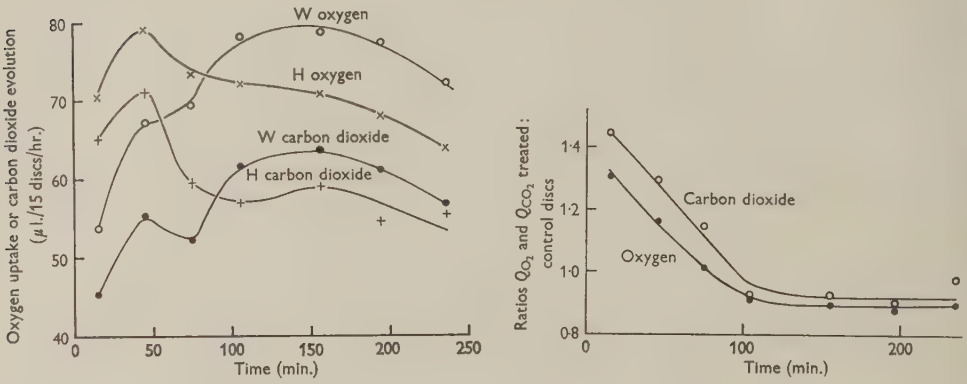
The herbicide usually decreased oxygen uptake as in Fig. 3, but sometimes it caused an initial stimulation and only later an inhibition. In an experiment on bean leaf discs treated for 18 hr. with diquat and then put for 1 hr. in dull light (Fig. 4*a*), the stimulation of oxygen consumption at the start of the period of measurement was paralleled by a stimulation of carbon-dioxide evolution. The ratios of both measured quantities for treated and control material declined to a constant level after some 2 hr. (Fig. 4*b*). In one experiment, on bean root tips treated with a 10^{-3} M solution of the herbicide in the dark, the stimulation of oxygen uptake continued throughout the whole of the measurement period (Fig. 5*a*). Even so, the ratio of oxygen consumption of treated to untreated roots fell and reached a steady value in about 200 min. (Fig. 5*b*).

The increase in both carbon-dioxide evolution and oxygen uptake suggests that the herbicide affects respiration. With bean and mustard leaf discs the herbicide

had only a small effect on the respiratory quotient; values for the experiment illustrated in Fig. 4*a* were:

Time (min.) ...	15	45	75	105	155	195	230
R.Q. control	0·84	0·81	0·75	0·79	0·81	0·78	0·79
R.Q. treated	0·93	0·89	0·81	0·79	0·83	0·81	0·87

The respiratory quotient of the treated discs differed from that of the controls only initially; the higher values indicating that carbon-dioxide evolution was stimulated relatively more than oxygen uptake. Thus there are strong reasons for supposing that there was also a stimulation of respiration in other experiments when the oxygen uptake only was measured, and found to be increased.



a *b*

Fig. 4*a*, *b*. Effect of the herbicide on respiration of bean discs. The discs were treated with water (W) or 10^{-5}M herbicide (H) for 18 hr. in the dark and 1 hr. in the light before measurements were made. The first readings were taken 85 min. after removal from the treating solutions.

The respiratory quotients of treated broad-bean root tips showed a larger and more persistent effect of the herbicide; root tips treated for 18 hr. with a 10^{-3}M solution of diquat and with a rate of oxygen evolution less than half that of the control gave the following results:

Time (min.) ...	20	40	80	140	210
R.Q. control	0·70	0·76	0·81	0·86	0·81
R.Q. treated	0·50	0·46	0·73	0·76	0·67

The low respiratory quotients of the treated roots, particularly in the first hour of measurement, possibly reflect an oxygen uptake associated with formation of black pigment greater than that associated with respiration. Bean leaf discs, unlike the roots, did not readily blacken in the dark (p. 604) and they would not therefore be expected to show this additional oxygen uptake.

The effect of oxygen on herbicidal activity in the light

Broad-bean leaf discs blackened less rapidly when they had sunk into the treating solutions. Moreover, if discs were deliberately injected by repeatedly evacuating the vessels containing the solutions on which the discs were floating, the onset of blackening was completely inhibited for several hours. Manometric experiments showed that the effect of the herbicide on oxygen consumption was also inhibited. For example, bean discs floated on a 10^{-3}M solution for 2 hr. in the dark, and then put in the light for another hour, gave an oxygen uptake of 28 μl./15 discs/hr., compared with 56 μl./15 discs/hr. for water-treated discs. When, however, the discs were injected immediately before transfer to the light the oxygen uptake was

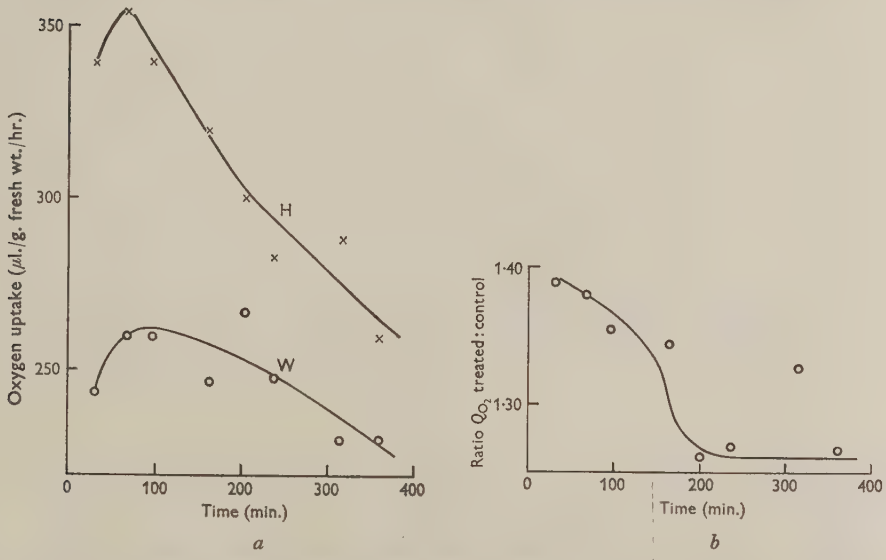


Fig. 5*a*, *b*. Effect of the herbicide on respiration of bean root tips. The roots were treated with water (W) or 10^{-3}M herbicide (H) for 16 hr. in the dark before measurements were made. The first readings were taken 150 min. after removal from the treating solutions.

subsequently 54 μl./15 discs/hr. Injection had no effect on the oxygen consumption of the controls. With mustard-leaf discs also, injection inhibited herbicidal action.

One consquence of injection was to reduce the availability of oxygen to the tissues, and herbicidal activity in the light was similarly inhibited by putting discs in nitrogen. In two more detailed experiments mustard-leaf discs were treated with 10^{-3}M diquat for 18 hr. in the dark, washed, put on water in contact with a range of oxygen concentrations, obtained by diluting air with nitrogen at atmospheric pressure, and left for an hour to attain equilibrium with the gas mixtures. After

this they were put in the light for 30 min., and finally transferred to the manometer flasks. A further set of treated discs was left in air in the dark, and the stimulation of herbicidal activity by light was measured by the difference in oxygen uptake of this dark set and the discs put in the light. These experiments showed that the oxygen level for the maximum light effect had to be at or near the atmospheric concentration (Fig. 6). Similar experiments with broad-bean discs gave more variable results, and the oxygen level needed to give the maximum light effect could not be determined accurately, although it appeared to be lower than in the mustard experiments.

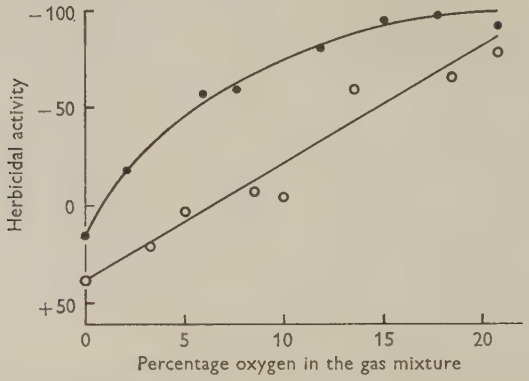


Fig. 6. The effect of oxygen concentration on herbicidal activity in the light. Details of the treatments are given in the text. The two curves are constructed from the results of two separate experiments. Herbicidal activity in the light was measured by depression of oxygen uptake, and increasing negative values on the ordinate indicate increasing activity.

Removal of oxygen had an almost immediate effect on herbicidal activity in the light. To demonstrate this, broad-bean discs were treated in the dark with 10^{-3}M diquat and then put on moist filter-paper in a flask from which the air was evacuated. One minute after the start of evacuation the flask was put in the light. The discs within failed to blacken, although others similarly treated but not evacuated darkened within 10 min. The evacuated flask was next put back in the dark and air readmitted, but the discs still remained green. If the herbicide had damaged the tissues in the light but pigment formation had been inhibited by evacuation, the discs would have been expected to go black in air on return to the dark. After 48 hr. in the dark the discs were put back in the light, this time without evacuation. They then blackened normally.

It must be emphasized that in all these experiments it was the effect of oxygen on herbicidal activity in the light that was studied. There are no comparable data to show whether oxygen is necessary in the dark.

Discussion

The rate of action of diquat was enhanced by light in all but one of the species tested. In the broad bean, but less obviously in other plants, the response differed qualitatively as well as quantitatively, the characteristic reaction in the light being the rapid formation of a black pigment in the leaves. This might suggest distinct modes of herbicidal action in light and dark, but these differences could have followed similar reactions to the herbicide occurring at different sites within the cell.

In the bean light affected only the green shoots. This, together with the failure of etiolated wheat to respond to light, and the inhibition of herbicidal activity in green tissues by monuron, supports the hypothesis that a reaction occurring during photosynthesis was essential to obtain the more rapid response. This is compatible with the chemical evidence that the herbicide is reduced to a free radical in the plant, because photosynthesis, through the photolysis of water, makes available a large reducing potential in green cells. It is supposed that diquat is reduced in the light as well as, or in place of, the normal photosynthetic reductants, and that death is more rapid than in the dark because this more intense reducing potential increases the rate of radical formation. This reasoning is further supported by the findings that carbon dioxide was not essential for the light effect, and that cyanide did not inhibit herbicidal activity. Therefore it is unlikely that a photosynthetic carbon product has to be formed to increase the rate of response of the plant.

If free radical formation is necessary for herbicidal action in the dark, or in tissues without chlorophyll, some process other than photosynthesis must effect the reduction of the herbicide. The manometric experiments suggest that this reduction is associated with respiration. The action of diquat on respiration was twofold: most experiments demonstrated an inhibition, and because this occurred particularly rapidly in green tissues exposed to light, it was probably brought about by reactions following the formation of radicals. In some experiments, however, there was clear evidence for a stimulation of respiration and this may indicate that the herbicide is reduced, particularly because the oxidation-reduction potential of -349 MV. (Homer & Tomlinson, 1959) is close to the potentials known to be reached during respiration.

The manometric measurements were always made after taking the material out of the treating solutions, and the level of respiration of the treated tissues in relation to the control commonly fell rapidly at first (Figs. 3*b*–5*b*). This fall was probably caused by the disappearance of a stimulating factor rather than by inhibition of respiration, because, in the experiments illustrated, no such inhibition had developed during the long treatment period. Evidently the stimulant may have been the unreduced herbicide, whose concentration in the tissues must have declined during the readings if it participated in irreversible reactions within the cells. On this interpretation the final flat part of curves 3*b* to 5*b* represents the more permanent effect of the herbicide on respiration. Fig. 4*b*, for example, shows that this was a small inhibition, which had at first been obscured by the larger stimulating effect of the remaining unreduced compound.

Although the formation of free radicals is essential to the sequence of toxic reactions, the experiments with oxygen indicate that this step alone is insufficient to kill the plant. Although photosynthesis in the intact cell is inhibited in plants kept anaerobically in the dark, this inhibition develops slowly (Rabinowitch, 1945, 1956) and cannot be obtained when even very small concentrations of oxygen are available (van der Veen, 1949; Warburg, 1958). Both these features contrast with the inhibition of herbicidal activity, which was rapidly established and, in mustard, began as soon as the oxygen concentration fell below atmospheric. Consequently there is no evidence that the photolysis reaction was inhibited in the experiments described here and, this being so, the conditions for radical formation would have been favourable even though there was no herbicidal activity. In the absence of oxygen it must be supposed that radicals formed in the light were harmlessly detoxified.

R. C. Brian (personal communication) drew attention to the similarity between the effect of oxygen on the action of diquat and its effect on the toxicity of ionizing radiations. These also induce radical formation, and in the presence of oxygen it is believed that peroxide radicals are the toxic agents. Some experiments, not reported here, were designed to test the hypothesis that peroxides are formed in tissues treated with the herbicide, but difficulties of interpretation have so far made it impossible to decide this question. As to the ultimate reason for the death of the plant there is no evidence, but it is not unlikely that if free radical chain reactions are begun, proteins and other large molecules in the protoplasm will be degraded. Such an occurrence would be consistent with the nature and rapid appearance of the visible toxic symptoms in treated plants.

Thanks are due to the author's colleagues at Jealott's Hill, especially R. C. Brian and T. E. Tomlinson, for many discussions held in the course of this work, and to Mrs P. Harlock for considerable technical assistance.

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METABOLISM OF PLANT GROWTH REGULATORS

I. 2,4-DICHLOROPHENOXYACETIC ACID IN LEAVES OF RED AND OF BLACK CURRANT

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(With Plate 9 and 3 Text-figures)

2,4-Dichlorophenoxyacetic acid (2,4-D) labelled with ^{14}C in either the carboxyl or methylene group was fed through the transpiration stream into detached leaves of black currant, which is sensitive to this herbicide, and of red currant which is highly resistant. The leaves were then kept in darkness and the evolution of $^{14}CO_2$ was followed; subsequently the leaves were extracted and the metabolic products examined by chromatography and radioassay.

The chief difference between the species is the ability of the red-currant leaf to oxidize approximately 50 % of the carboxyl and 20 % of the methylene carbon from the side chain of the 2,4-D over a period of 1 week: in the black-currant leaf only about 2 % of the 2,4-D is broken down in this way in the same period. The red-currant leaf can metabolize in a similar manner the side chains of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 4-chlorophenoxyacetic acid (4-CPA) and 2-methyl-4-chlorophenoxyacetic acid (MCPA), but not of 2-chlorophenoxyacetic acid (2-CPA).

In both species 5–10 % of the 2,4-D taken up by the leaves is converted to water-soluble compounds which are biologically inactive in the oat mesocotyl and *Coleus* abscission tests and which yield 2,4-D on hydrolysis with 2N sulphuric acid. The failure of 2-CPA to produce growth responses in currants, and probably in other plants, is attributable to the conversion of 70–80 % of the applied chemical to water-soluble inactive compounds. A further 5–10 % of the 2,4-D entering the leaf is metabolized to a biologically active, non-acidic neutral compound. Analogous compounds were formed in leaves treated with 2,4,5-T or 2-CPA.

In both species a variable proportion (10–30 %) of 2,4-D, 2,4,5-T and 2-CPA are bound in the leaf tissue and cannot be removed by aqueous or organic solvents or by mild hydrolysis.

The red currant is killed by low concentrations of 2,4,5-T even though this compound is broken down in the leaf as rapidly as 2,4-D. It is noteworthy that death is delayed and there is an absence of the epinastic reactions normally associated with damage by phenoxy-acid herbicides.

Introduction

Field studies on the use of herbicides in fruit crops (Luckwill & Campbell, 1957) showed that whereas black currant (*Ribes nigrum* L.) is very susceptible to damage by 2,4-D, the red currant (*R. sativum* Syme) is highly resistant. The experiments described in this paper deal with the metabolism of 2,4-D in detached leaves of these two species. They were undertaken to discover the reason for the difference in their reaction to this herbicide, and to contribute to a better understanding of the broader aspects of selective toxicity as shown by the phenoxy-acid herbicides.

Previous studies of this type (Gallup & Gustafson, 1952; Fang & Butts, 1954; Weintraub, Reinhart & Scherff, 1956) have been concerned primarily with comparisons of the penetration and translocation of radioactive 2,4-D in susceptible and resistant species, though Jaworski & Butts (1952) and Fang & Butts (1954) also made a preliminary study of the breakdown products of 2,4-D in beans and wheat. The plants they worked with, however, differed so widely in structure and physiology that precise comparisons of their metabolic processes was not possible.

Methods

Treatment of leaves. The primary object was to compare quantitatively the metabolism of 2,4-D within the leaf tissues of closely related species. A technique was therefore chosen which would eliminate differences due to penetration and translocation of the herbicide and which would at the same time permit measurement of the amount of 2,4-D which entered the tissues. Detached leaves, immediately after severing from the plant were stood with their petioles dipping into small vials of an aqueous solution of 2,4-D labelled with ^{14}C in either the carboxyl or methylene group.* During a period of 3 hr. in the light the leaves took up between 2 and 4 ml. of solution, normally of 20 p.p.m., through the transpiration stream. The uptake was determined by the loss in weight of the vial and solution, evaporation being reduced by fitting each vial with a foil cap through which the petiole passed.

Investigation of metabolic products. After the growth regulator was introduced, the leaves were transferred to closed jars and left with their petioles dipping into water for periods of up to 163 hr. During this time they were kept in darkness at 25° C. Carbon dioxide-free air was passed into the jars, and the carbon dioxide liberated by the leaves was collected in Pettenkoffer tubes containing barium hydroxide solution and estimated gravimetrically as barium carbonate. The radioactivity of the precipitated carbonate was measured under an end-window Geiger-Müller tube (type EW.3H, 1·5–2·5 mg./cm.^{2} mica window).

The 2,4-D remaining in the leaf after varying periods in darkness, and the metabolic products formed from it, were extracted by maceration in 2% sodium bicarbonate (Andreae & Good, 1955) or very dilute ammonium hydroxide (pH 11); the latter solvent was preferable when the extract was subsequently to be concentrated for chromatography. The aqueous extract was clarified by filtration, acidified to pH 3, and shaken with methylene chloride. Three shakings of the aqueous extract, each with an equal volume of methylene chloride, were found to be sufficient to remove all the unchanged 2,4-D from the extract. In some experiments ether was used in place of methylene chloride, but owing to its greater miscibility with water complete removal of 2,4-D from the aqueous phase was more difficult with this solvent.

The following is a typical extraction procedure: a leaf of 2·5 g. fresh weight was macerated for 5 min. in 10 ml. dilute ammonium hydroxide (pH 11). The macerate was allowed to stand, with frequent shaking, for 15 min. and after addition of

* Obtained from the U.K.A.E.A., Amersham, Bucks.

0·5 g. Kieselguhr was filtered through Whatman no. 4 paper in a Buchner funnel. The residue was washed four times on the paper with 8 ml. portions of dilute ammonia, 5 min. being allowed for each washing before sucking dry. The residue was removed from the paper and dried at 80° C. for 2 hr. The filtrate was acidified with dilute HCl to pH 3, and any precipitate was removed by centrifuging and added to the residue. The acidified filtrate was then shaken three times with an equal volume of methylene chloride. Frequently the mixture emulsified, and the two phases were separated by centrifuging at 2000 r.p.m. for 10 min. The methylene chloride phase was taken to small volume by distillation and finally reduced to dryness by drip distillation into a small test tube. The dry residue was taken up in 0·2 ml. of a mixture of methanol, chloroform and water (4:3:1). The volume of the aqueous phase was measured and a 2 ml. aliquot placed in a small open dish and left to evaporate to dryness at 25° C. The dry residue was dissolved in 0·1 ml. of water.

The total radioactivity in the organic and aqueous phases was estimated by spotting 30 μl. aliquots of the concentrated extracts on to filter-paper and counting under an end-window G-M tube, whilst the relative proportions of each of the various compounds in each phase were determined by radioassay in a 4π windowless Geiger counter (Harrison & Winteringham, 1955) after separation by ascending paper chromatography in a mixture of *iso*-propanol/water/ammonia (10:1:1).

Some of the chromatograms were also assayed for auxin activity by cutting them into 1 cm. lengths and testing each piece in the oat mesocotyl test (Nitsch & Nitsch, 1956) or the *Coleus* petiole abscission test (Luckwill, 1956).

Radioactivity remaining in the leaf tissue after extraction was estimated by combusting the oven-dried residue, precipitating the carbon dioxide as barium carbonate, and counting the filtered precipitate under the end-window G-M tube (Lindenbaum, Schubert & Armstrong, 1948).

Results

Distribution of 2,4-D *in the leaf*

Autoradiographs were made of red-currant leaves which had taken up carboxyl-labelled 2,4-D from a 20 p.p.m. solution, using the technique of Thomas, Bennett & Lloyd-Jones (1955). These showed that, at the end of the uptake period, the 2,4-D was predominantly in the vascular system. One hour later the main veins were already free of 2,4-D, and after 24 hr. all the radioactivity was located in the mesophyll (Pl. 9, fig. 1).

Response of red and black currants to 2,4-D

Actively growing shoots were cut and totally immersed for 3 sec. in solutions of 2,4-D of different concentrations to which had been added 0·025% of a non-ionic wetting agent. The shoots were then stood in water for 1 week before recording. The striking difference in response of these two species to 2,4-D is shown in Pl. 9, fig. 2. Black currant (var. Baldwin) showed marked epinastic response to concentrations as low as 31 p.p.m., whereas with red currant (var. Laxton's no. 1) no

response was obtained with concentrations up to 250 p.p.m. One month after treatment all the black-currant shoots that had received 2,4-D in excess of 15 p.p.m. were dead, whereas none of the red-currant shoots showed signs of damage. The concentration of 2,4-D required to kill red-currant shoots within 1 month of treatment was found to be approximately 1500 p.p.m.

Decarboxylation of 2,4-D by detached leaves

Leaves of red currant (Laxton's no. 1) and black currant (Wellington XXX) collected in August from bushes growing in the field were used. Eight leaves of each species were treated with 10 p.p.m. carboxyl-labelled 2,4-D as already described, of which four were placed in respiration chambers for a period of 139 hr. These leaves were then divided longitudinally and the fresh weight of each half determined. The total radioactivity in one half of each leaf was measured whilst the other half was extracted and the extract subsequently shaken with ether. The other four leaves were similarly treated immediately at the end of the uptake period. The ether extracts on chromatographic analysis were found to contain 2,4-D as the chief radioactive component, as judged from its R_F value.

Table 1. *Recovery of ^{14}C of carboxyl-labelled 2,4-D from leaves of black currant and red currant*

	Time* (hr.)	2,4-D uptake (μg.)	% carboxyl carbon recovered		
			As CO_2	In ether phase	On leaf residue
Black currant	0	49·6	0	63	12
	139	55·7	2	56	7
Red currant	0	49·6	0	63	16
	139	44·6	48	13	22

* Times are measured from the end of the uptake period.

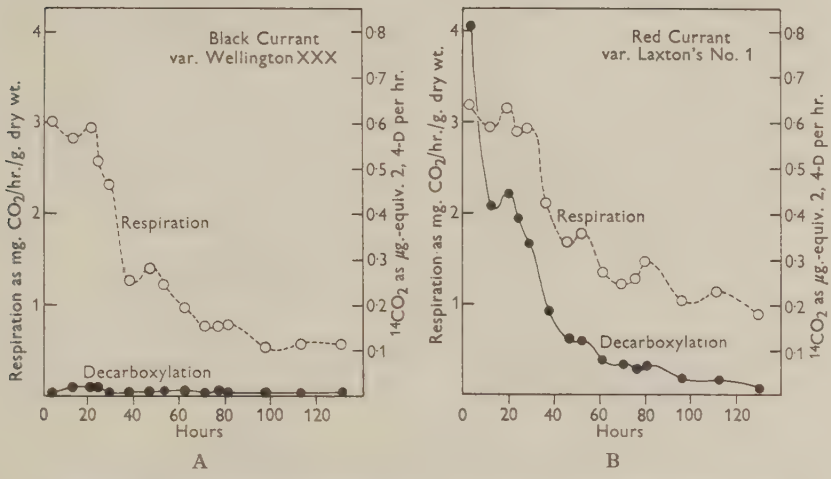
The experimental results (Table 1) show that in the susceptible black currant there was little reduction in the concentration of extractable 2,4-D over a period of 139 hr., whereas in the same period the concentration in the resistant red currant fell to about 20% of its initial level. It is clear that this difference is due chiefly to the ability of the red-currant leaves to break down the side chain of the 2,4-D molecule with the liberation of the labelled carboxyl carbon as carbon dioxide. The very low rate of decarboxylation in the black-currant leaves cannot be attributed to damage. There was no visible damage to the leaves by the 2,4-D and the course of respiration throughout the experiment was similar in both black and red currant (Text-fig. 1).

The red-currant leaf residue retained more radioactivity than the black. This bound 2,4-D was not removed by mild hydrolysis in 2N sodium hydroxide or 2N sulphuric acid or by Soxhlet extraction of the dried residue with ether, ethanol or acetone.

A further experiment was carried out in April using leaves of the same varieties

from bushes forced into early growth in the glasshouse. Four leaves of each species were fed with 20 p.p.m. carboxyl-labelled 2,4-D and transferred to respiration chambers for periods varying from 20 to 115 hr. The leaves were then extracted and analysed as described under Methods (p. 614).

The results of this experiment (Table 2) confirm the much greater rate of decarboxylation in the red currant and show that the amount of ^{14}C in the methylene chloride phase—mostly unmetabolized 2,4-D—falls correspondingly faster in the red as compared with the black currant. The amounts of ^{14}C in the aqueous phases were similar, as were also the amounts bound in the leaf residues.



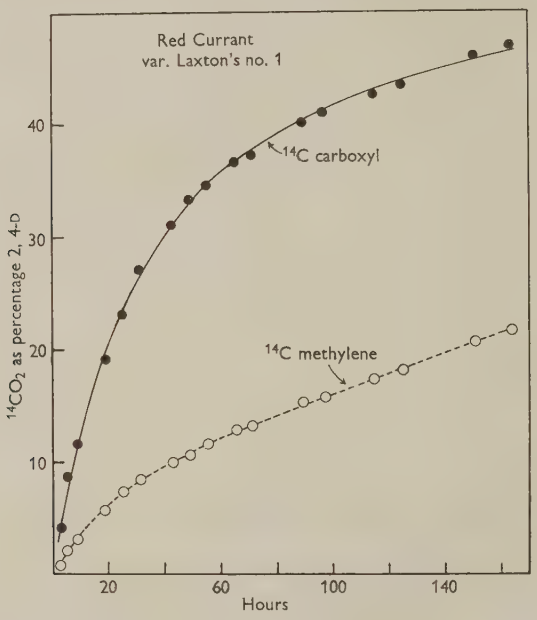
Text-fig. 1. Rate of decarboxylation of 2,4-D (solid line) and rate of respiration (broken line) in detached leaves of black currant (A) and red currant (B).

TABLE 2. *Recovery of carboxyl carbon from leaves of red and black currants at varying times after treatment with known amounts of* ^{14}C *carboxyl-labelled* 2,4-D

	Time (hr.)	2,4-D uptake (μg.)	% carboxyl carbon recovered: As CO_2	In CH_2Cl_2 phase	In aqueous phase	On leaf residue	Total
Black currant	20	46·0	0	91	2	13	106
	44	50·6	1	67	4	34	106
	67	58·2	3	58	4	31	96
	115	75·8	1	64	8	36	109
Red currant	20	45·5	7	67	3	35	112
	44	40·6	19	38	5	34	96
	67	56·2	31	22	6	34	92
	115	56·8	29	31	5	36	101

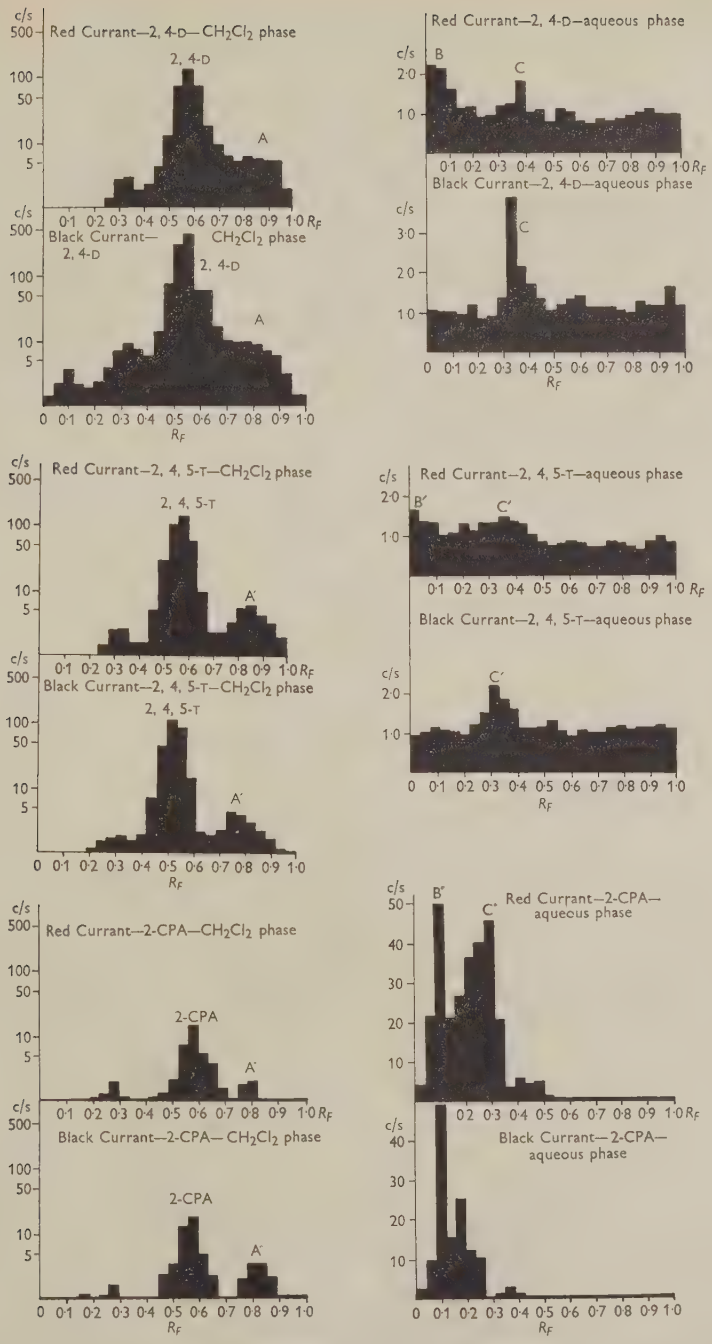
Chromatography of the leaf extracts

Radioassay of chromatograms of the methylene chloride phase showed two radioactive peaks, one, assumed to be 2,4-D, at R_F 0·5–0·7, and the other (compound A) at R_F 0·7–0·9 (Text-fig. 3). Compound A was found to pass readily from aqueous solution into methylene chloride even at pH 11, so that although it contains the original carboxyl carbon of 2,4-D it cannot be acidic in nature. Of the total radioactivity in the methylene chloride phase it was estimated that about 80% was 2,4-D and 20% was compound A. Indications of traces of a third radioactive compound (R_F 0·2–0·4) in the methylene chloride phase were also obtained.



Text-fig. 2. Carbon dioxide production from carboxyl and methylene carbon atoms of 2,4-D in leaves of red currant.

Chromatograms of the aqueous phase of the red-currant leaf extracts (Text-fig. 3) contained two radioactive compounds, at R_F 0–0·1 (compound B) and R_F 0·3–0·4 (compound C). These were present in almost equal amounts and together accounted for about 5% of the total ^{14}C recovered. The corresponding aqueous fractions from the black currant contained only compound C, except the 115 hr. sample in which a very small quantity of B was detected.



Text-fig. 3. Distribution of radioactivity (in counts per second) on chromatograms of methylene chloride and water phases of extracts of leaves treated with ^{14}C carboxyl-labelled 2,4-D, 2,4,5-T and 2-CPA.

Metabolism of the methylene carbon

Six leaves of red currant (var. Laxton's no. 1) were collected from field-grown bushes in October. Three leaves were treated with carboxyl-labelled 2,4-D and three with methylene-labelled 2,4-D. The leaves remained in the respiration chambers for 163 hr.

The results of this experiment (Text-fig. 2) show that carbon dioxide is also produced from the methylene carbon, but at less than half the rate of that from the carboxyl carbon. Chromatograms of the aqueous and methylene chloride phases were run and radioassay showed that the extracts of the carboxyl and methylene treated leaves contained the same labelled compounds in approximately the same amounts. No indication was found on these chromatograms of a compound containing only the methylene carbon.

Two leaves of the same variety of red currant were used in a further experiment, one being treated with methylene-labelled and the other with carboxyl-labelled 2,4-D. After 72 hr. in the respiration chambers the leaves were extracted as previously described.

Table 3. *Recovery of the methylene and carboxyl carbon of* 2,4-D *from leaves of red currant 72 hr. after treatment*

	Carboxyl-carbon		Methylene-carbon	
	μg.	%	μg.	%
2,4-D taken up	78·6	100	81·4	100
Lost as CO_2	20·9	27	9·4	12
CH_2Cl_2 phase (2,4-D + A)	27·1	35	22·5	28
Aqueous phase (B + C)	2·9	4	5·5	7
Bound on leaf residue	23·8	30	38·3	47
Total recovery	74·7	96	75·7	94

The analysis presented in Table 3 shows that the metabolic product of 2,4-D containing only the methylene carbon is located in the leaf residue, which contains 17% more methylene than carboxyl carbon. This corresponds closely to the difference between the carbon dioxide production from these two atoms of the side chain. Subsequent Soxhlet extraction of the dried residue of the methylene-treated leaves with ethanol and afterwards with hexane, failed to remove any significant radioactivity.

Properties of the water-soluble metabolic products

The nature of the unknown compounds B and C was investigated by hydrolysis of the appropriate zones of the chromatogram paper with 2N sulphuric acid for 5 hr. Compound B was converted into a methylene chloride-soluble compound which from its R_F value was assumed to be 2,4-D. Hydrolysis of C under similar conditions gave a methylene chloride soluble compound (D) of R_F 0·1–0·2. Further hydrolysis converted compound D into 2,4-D.

Unlike compound A, which was highly active in both the oat mesocotyl and *Coleus* petiole abscission tests, compounds B and C showed no auxin activity.

Response of currant shoots to other substituted phenoxy-acids

Shoots of red currant (var. Laxton's no. 1) and black currant (var. Wellington XXX) about 30 cm. long were collected from young vigorous bushes in July. The shoots were thoroughly wetted with aqueous solutions of five substituted phenoxy-acids at concentrations of 10, 30, 100, 300 and 1000 p.p.m. The solutions contained a non-ionic wetting agent, and control shoots, treated with a solution of the wetting agent, were included. After 1 month records were taken of the lowest concentration of each compound required to cause damage to the shoots and of the lowest concentration that resulted in death (Table 4).

TABLE 4. *Lowest concentration (in p.p.m.) of various substituted phenoxy-acids which cause damage or death of cut shoots of red and black currant within one month of application*

	Black currant		Red currant	
Compound	Damaged by	Killed by	Damaged by	Killed by
2,4-D	—	10	1000	> 1000
2,4,5-T	—	10	—	10
2-CPA	> 1000	> 1000	> 1000	> 1000
4-CPA	100	300	1000	> 1000
MCPA	30	100	100	> 1000

The difference in the susceptibility of the red and black currant to 2,4-D was even greater in this than in previous experiments, black currant being killed by 10 p.p.m. whilst red currant survived 1000 p.p.m. Both species, however, proved equally susceptible to 2,4,5-T, being killed by the lowest concentration tested (10 p.p.m.), though there was a marked difference in their reactions to this compound. Within 1–2 days of treatment the black-currant shoots showed violent epinastic reactions and twisting of the stem; death occurred from the tip downwards. The red currant appeared undamaged up to 1 week after treatment, but after this period there was a progressive yellowing of the foliage from the base of the shoot upwards, and this yellowing was followed by death.

The reaction to the 2-chlorophenoxyacetic acid was unexpected; although this compound shows some biological activity at 100 p.p.m. in the coleoptile cylinder test, the pea test and the tomato epinasty test (Fawcett, Wain & Wightman, 1955) it caused no damage to either species of currant at concentrations of up to 1000 p.p.m. Black currant was more readily damaged by 4-chlorophenoxyacetic acid and MCPA than was red currant, but the difference between the species was less marked than with 2,4-D.

Decarboxylation of other phenoxy-acids by currant leaves

Leaves of red and black currant (Laxton's no. 1 and Wellington XXX) were allowed to take up 20 p.p.m. solutions of five carboxyl-labelled phenoxy acids as previously described (p. 614) and were then transferred to respiration chambers for 20 hr. Red currant decarboxylated all the acids except 2-chlorophenoxyacetic acid.

By contrast no radioactivity was detected in the carbon dioxide given off by the black-currant leaves (Table 5).

In the experiment summarized in Table 6 pairs of black-currant leaves (var. Baldwin) and red-currant leaves (var. Red Lake) were fed with ^{14}C carboxyl-labelled 2,4-D, 2,4,5-T and 2-CPA and, after 91 hr. in the respiration chambers, the leaves were extracted and analysed.

Table 5. *Decarboxylation of five different substituted phenoxy-acids by leaves of black and red currant over a period of 20 hr. in darkness at 25° C.*

	Black currant		Red currant	
Compound	Uptake (μg.)	% decarboxylated	Uptake (μg.)	% decarboxylated
2,4-D	62·6	0	71·2	16
2,4,5-T	63·2	0	28·4	25
2-CPA	47·8	0	42·2	1
4-CPA	53·2	0	38·2	12
MCPA	60·6	0	56·4	12

Table 6. *Recovery of carboxyl carbon from leaves of black and red currant 91 hr. after treatment with three different ^{14}C carboxyl-labelled phenoxy-acids*

			% carboxyl carbon recovered				
Species	Compound	Uptake (μg.)	As CO_2	In CH_2Cl_2 phase	In aqueous phase	On leaf residue	Total
Black currant	2,4-D	113·8	1	70	7	13	91
	2,4,5-T	61·0	0	66	10	14	90
	2-CPA	76·0	0	5	71	20	96
Red currant	2,4-D	87·4	27	28	6	19	80
	2,4,5-T	82·2	20	58	9	19	105
	2-CPA	84·0	0	2	81	17	100

The results indicate that the metabolism of 2,4,5-T in red- and black-currant leaves follows closely the pattern already established for 2,4-D. The behaviour of 2-CPA is different in two respects: first, as found earlier, there was no decarboxylation of this compound by either species; and secondly, the proportion of the applied compound converted into water-soluble substances is about ten times greater than for 2,4-D or 2,4,5-T.

The leaf extracts in this experiment, after separation into methylene chloride and aqueous phases, were chromatogrammed on paper. For all three compounds the methylene chloride phase was found to contain a radioactive compound (A) of higher R_F value than the original acid. The aqueous phases from red currant all contained two radioactive compounds (B and C) of which only one was present in black currant (Text-fig. 3).

Discussion

The results of these experiments indicate the sequence of events which follows the introduction of 2,4-D into detached leaves of black and red currant. In both species the concentration of 2,4-D in the leaf falls continuously over a period of 80–100 hr., but in the resistant red currant the fall is much more rapid than in the sensitive black currant. Three mechanisms are involved in this loss of 2,4-D:

(1) *Breakdown of the side chain*

The red currant differs from the black currant in its ability to oxidize the side chain of the 2,4-D molecule. The fact that carbon dioxide is produced from the carboxyl carbon over twice as fast as from the methylene carbon implies the accumulation of an intermediate containing the methylene but not the carboxyl carbon of the 2,4-D molecule. This intermediate, which could be 2,4-dichloroanisole, is bound on the leaf residue and attempts to extract it were not successful.

The red currant is also able to decarboxylate 2,4,5-T, 4-CPA and MCPA. Its apparent inability to decarboxylate 2-CPA may be due to the fact that virtually all the compound introduced into the leaf is either bound or metabolized to water-soluble substances.

Weintraub *et al.* (1952), studying 2,4-D metabolism in *Phaseolus vulgaris*, have previously reported the oxidation of 5% of the carboxyl and 3% of the methylene carbon to carbon dioxide over a period of 4 days. These values are comparable with those found for black currant and are of quite a different order from those for red currant, which are 42 and 16% respectively.

(2) *Formation of metabolic products*

5–10% of the 2,4-D entering the leaf of red or black currant is converted into water-soluble compounds (B and C) which are insoluble in ether and methylene chloride, but which yield 2,4-D on acid hydrolysis. Similar compounds have been reported by Jaworski & Butts (1952), who suggested that one of their unidentified compounds might be a glucoside of 2,4-D, and by Andreae & Good (1957) who identified 2,4-dichlorophenoxyacetoaspartic acid as a metabolic product of 2,4-D in peas. Compound B was found to have the same R_F value in *iso*propanol as synthetic 2,4-dichlorophenoxyacetoaspartic acid. However, Krewson *et al.* (1954) have synthesized many other products of amino acids with substituted phenoxyacetic acids, and it is possible that certain of these might also be formed naturally in plants treated with 2,4-D.

Since compounds B and C have no auxin activity their formation may be regarded as a detoxication mechanism which, whilst of minor importance for 2,4-D and 2,4,5-T, assumes greater significance with 2-CPA, 70–80% of which is inactivated in this way. The extent of this conversion, together with binding in the leaf tissue, quickly reduces the amount of 2-CPA in the leaf to 1–2% of that applied, which may well explain the inactivity of this compound on currants and its low

activity in auxin tests as reported by Muir, Hansch & Gallup (1949), Leaper & Bishop (1951), and Fawcett *et al.* (1955).

About 16% of the 2,4-D entering the leaf of black or red currant is converted into a biologically active non-acidic compound of higher R_F value (A). Analogous compounds were identified in the extracts of leaves treated with 2,4,5-T and 2-CPA.

(3) *Binding*

In both species 10–30% of the applied 2,4-D is bound in the tissues of the leaf in such a way that it cannot be removed by solvent extraction or mild hydrolysis. Binding is complete about 20 hr. after treatment and the 2,4-D thus held is not subsequently released or further metabolized. Binding may involve the formation of 2,4-D-protein complexes similar to the IAA-protein complex found by Siegel & Galston (1953) in peas and the 2,4-D-protein complex which Butts & Fang (1956) claim to have identified in beans.

The experiments described in this paper do not indicate why 2,4-D is toxic to black currant. They do, however, suggest that the toxic action is associated with the presence of free 2,4-D in the tissues and that the resistance of the red currant is due chiefly to its ability to detoxicate the molecule by oxidation of the side chain.

The authors gratefully acknowledge the help of Mr R. G. Powell of the A.R.C. Unit of Experimental Agronomy, Oxford, who supplied samples of ^{14}C carboxyl-labelled 2,4,5-T, 2-CPA, 4-CPA and MCPA, and of Dr D. Woodcock, Long Ashton, who synthesized the 2,4-dichlorophenoxyacetoaspartic acid. They also thank Mr P. Weaver, Miss M. R. Manning and Miss J. Parsons for technical assistance.

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Fig. 1 A

Fig. 1 B

Fig. 1 C

Fig. 2 A

Fig. 2 B

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EXPLANATION OF PLATE 9

Fig. 1. Autoradiographs of red-currant leaves fed through the petioles with ^{14}C carboxyl-labelled 2,4-D. A = at end of uptake period—2,4-D in vascular system; B = 1 hr. later—2,4-D has moved from the principal veins; C = 24 hr. later—2,4-D is in the mesophyll.

Fig. 2. Shoots of black currant (A) and red currant (B) 1 week after dipping in 2,4-D solutions of concentrations 0–250 p.p.m.

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METABOLISM OF PLANT GROWTH REGULATORS

II. DECARBOXYLATION OF 2,4-DICHLOROPHENOXYACETIC ACID IN LEAVES OF APPLE AND STRAWBERRY

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(With Plate 10 and 5 Text-figures)

Comparison of the metabolism of ^{14}C carboxyl-labelled 2,4-D in detached leaves of different varieties of apple showed that the 2,4-D-resistant Cox decarboxylated 57 % of the 2,4-D in the leaf in 92 hr., whereas the susceptible Bramley's Seedling decarboxylated only 2 % in the same time. Comparatively high rates of decarboxylation were found with all varieties of apple having Cox in their parentage; varieties unrelated to Cox showed low rates of decarboxylation. Similar high rates of decarboxylation were found in resistant varieties of strawberry and in *Syringa vulgaris*. Sixteen other species tested showed low rates of decarboxylation, irrespective of whether they were resistant or susceptible to 2,4-D.

The rate of decarboxylation of 2,4-D in the mature strawberry leaf was proportional to the amount of 2,4-D in the leaf. The rapid fall in decarboxylation rate in a detached leaf was due chiefly to reduction in the amount of 2,4-D present, and not to any falling off in the metabolic activity of the leaf. Young leaves decarboxylated 2,4-D at about half the rate of fully grown or senescent leaves. Oxidation of the methylene carbon proceeds at less than half the rate of that of the carboxyl carbon and is accompanied by an increase in the phenol content of the leaf which agrees closely with the amount of 2,4-D demethylated.

As previously reported for currants, part of the 2,4-D entering the leaf was bound and part inactivated by the formation of water-soluble compounds. The reaction of strawberry variety Talisman to 2,4,5-trichlorophenoxyacetic (2,4,5-T), 2-chlorophenoxyacetic (2-CPA), 4-chlorophenoxyacetic (4-CPA) and 2-methyl-4-chlorophenoxyacetic (MCPA) acids was similar to that previously reported for red currant. Leaves of Cox's Orange Pippin apple and Talisman strawberry were able to decarboxylate all these phenoxy-acids except 2-CPA.

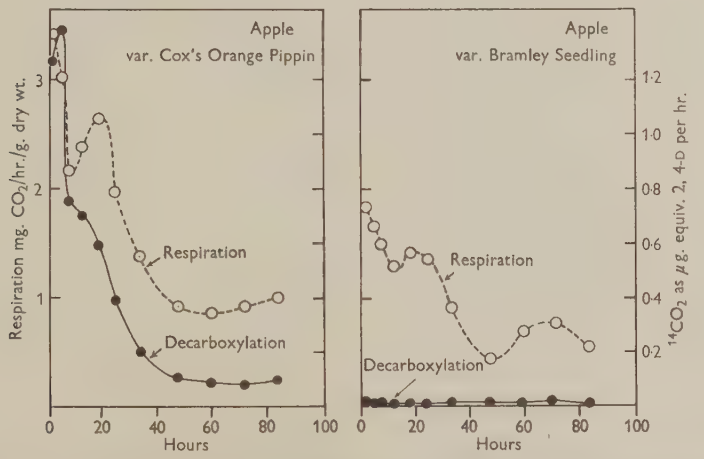
Introduction

In a previous paper (Luckwill & Lloyd-Jones, 1960) it was shown that the resistance of the red currant to herbicidal applications of 2,4-D is associated with its ability to rapidly decarboxylate the side chain of this acid with the liberation of carbon dioxide. The closely related black currant, which is readily killed by low concentrations of 2,4-D, does not possess this ability. This paper presents further studies of the metabolism of 2,4-D in detached leaves of other resistant and susceptible species, with particular emphasis on decarboxylation. The methods used have been fully described in the first paper of this series.

Experimental

Reaction of apple varieties to 2,4-D

The reaction of apple varieties to 2,4-D varies from high sensitivity to almost complete resistance (Luckwill & Campbell, 1957). Bramley's Seedling was chosen as an example of a 2,4-D-sensitive variety and Cox's Orange Pippin as a resistant variety, their contrasting reactions being illustrated in Pl. 10, fig. 1). Six weeks after spraying with a 30 p.p.m. solution of 2,4-D, Bramley's Seedling showed marked stunting and spectacular formulative effects on the terminal growth, whilst higher concentrations caused death of the shoot tip and complete suppression of growth. Trees of Cox's Orange Pippin sprayed at the same time showed no reaction to sprays of up to 500 p.p.m. 2,4-D.



Text-fig. 1. Decarboxylation of carboxyl-labelled 2,4-D (solid lines) by leaves of Bramley's Seedling and Cox's Orange Pippin apples in darkness at 25° C. The respiration rates of the leaves are shown by the broken lines.

Decarboxylation of 2,4-D *by Cox and Bramley*

100 μg. ^{14}C carboxyl-labelled 2,4-D as a 20 p.p.m. aqueous solution was fed through the petiole into four leaves of Cox and a similar amount into four of Bramley. The leaves were then transferred to respiration chambers for 92 hr., during which time frequent measurements of respiration and evolution of radioactive carbon dioxide were made. Subsequently the leaves were extracted and the radioactive metabolic products investigated.

Cox initially showed a high rate of decarboxylation which fell rapidly over a period of 40 hr. and thereafter more slowly (Text-fig. 1); Bramley showed a very slow but steady rate of decarboxylation over the whole period of the experiment. After 92 hr. 57% of the 2,4-D in the Cox leaves had been decarboxylated compared

with only 2% in Bramley. A further 28% of the 2,4-D in the Cox leaves was in bound form and 13% present as inactive water-soluble compounds; less than 2% was still present as free 2,4-D.

Decarboxylation of 2,4-D by other apple varieties

Carboxyl-labelled 2,4-D was fed into the leaves of twenty-four varieties of apple and the percentage of the 2,4-D decarboxylated over a period of 20 hr. at 25° C. in darkness was measured (Table 1).

Table 1. *Decarboxylation of 2,4-D by detached leaves of apple varieties and rootstocks over a period of 20 hr. in darkness at 25° C.*

Variety	2,4-D uptake (μg.)	% decarboxylated in 20 hr.
*Laxton's Fortune	30·4	28·4
Cox's Orange Pippin	31·0	25·6
*Ellison's Orange	36·2	12·6
*Laxton's Epicure	38·0	12·6
*Tydeman's Late Orange	40·6	11·3
*Allington Pippin	27·6	10·5
*Rival	32·2	10·2
†Ribston Pippin	31·8	9·4
*Laxton's Superb	36·6	8·5
*Merton Worcester	28·8	8·2
*Merton Prolific	38·0	7·1
Worcester Pearmain	33·2	5·9
Malling IX rootstock	28·6	5·5
Lord Lambourne	57·8	5·5
Malling VII rootstock	36·8	5·4
Jonathan	37·2	3·8
M.M. 106 rootstock	37·8	3·0
Beauty of Bath	48·6	2·5
Malling II rootstock	24·2	0·7
Crab C rootstock	28·8	0·5
Bramley's Seedling	33·6	0·5
M.M. 104 rootstock	42·4	0·3
Miller's Seedling	30·6	0·3
Emneth Early	58·8	0·2

* Seedling of Cox's Orange Pippin. † Parent of Cox's Orange Pippin.

Only one other variety was found with the ability to decarboxylate 2,4-D as rapidly as Cox's Orange Pippin. This was Laxton's Fortune, which originated as a seedling of Cox. However, comparatively high rates of decarboxylation were shown by all those varieties having Cox in their parentage, and also by Ribston Pippin, which is the female parent of Cox. Conversely, it was found that those varieties unrelated to Cox had low rates of decarboxylation (less than 6% in 20 hr.). There is a clear indication here that ability to decarboxylate 2,4-D may be an inherited character.

Reaction of strawberry varieties to phenoxy-acids

Potted plants of eight varieties of strawberry were sprayed to run-off with solutions of 2,4-D sodium salt at 100, 300 and 1000 and 3000 p.p.m. The sprays contained a non-ionic wetting agent and controls were sprayed with water containing the same concentration of wetting agent. There were three replicate plants per treatment. The varieties used were: Royal Sovereign, Talisman, Redgauntlet, Huxley, Cambridge Favourite, Cambridge Rearguard, Cambridge Vigour and Madame Lefebvre. The plants were sprayed on 25 May and kept in a greenhouse until the middle of August.

On most varieties these sprays caused remarkably little damage. Plants sprayed with 1000 and 3000 p.p.m. produced a few 'frenched' leaves, and those receiving the highest concentration appeared slightly retarded in growth and runner production. The only variety to show severe damage was Huxley, the growth of which was strongly suppressed by the 3000 p.p.m. and slightly suppressed by the 1000 p.p.m. spray. In general it appears that strawberries possess a very high degree of resistance to 2,4-D and that varietal differences in susceptibility are much less marked than in apples.

In a further resistance trial, plants of the strawberry variety Talisman were sprayed with five different phenoxy-acids at concentrations of 250, 500, 1000 and 2000 p.p.m. in water. The plants were photographed 3 months after spraying (Pl. 10, fig. 2). Although Talisman showed almost complete resistance to 2,4-D and 2-CPA, it was severely damaged by the higher concentrations of 2,4,5-T and MCPA and, to a lesser extent by 4-CPA. A characteristic feature of the damage, especially that caused by 2,4,5-T, was the yellowing of older leaves, which became marked about one week after spraying.

Oxidation of the 2,4-D side chain by strawberry

Approximately 60 μg. carboxyl-labelled 2,4-D was fed into leaves of three varieties of strawberry, two of which (Royal Sovereign and Cambridge Favourite) had shown high resistance to 2,4-D and one of which (Huxley) was somewhat more susceptible. Over a period of 80 hr. Royal Sovereign and Cambridge Favourite decarboxylated 60%, and Huxley 48%, of the applied 2,4-D. These results suggest that in strawberries as in currants and apples, resistance to 2,4-D is associated with high rates of decarboxylation of the acid.

In two further experiments the oxidation of the methylene carbon as well as the carboxyl carbon of the side chain was studied. Leaves of Talisman strawberry were fed with either carboxyl- or methylene-labelled 2,4-D and the production of radioactive carbon dioxide was followed for periods of 67 and 115 hr. At the end of these periods the distribution of radioactivity remaining in the leaf was investigated.

The results of these two experiments, which are in close agreement (Table 2), show that approximately 50% of the 2,4-D is decarboxylated but only about 20% is demethylated. It would therefore be expected that approximately 30% of the

2,4-D would have been converted into a compound containing the methylene but not the carboxyl carbon of the original 2,4-D molecule. As was found in red currant (Luckwill & Lloyd-Jones, 1960), this intermediate in the breakdown of the side chain is present in non-extractable form in the leaf residue.

Table 2. *Recovery of carboxyl and methylene carbon of 2,4-D in leaves of strawberry var. Talisman*

Date	Time (hr.)	^{14}C label	2,4-D uptake (μg.)	% C or M carbon recovered: As CO_2	In CH_2Cl_2 phase	In aqueous phase	On leaf residue
Oct.	115	C	103·4	51	4	18*	27
		M	104·0	21	5	15*	59
June	67	C	115·0	47	7	12	28
		M	105·4	18	5	14	50

* Estimated by difference.

Increase in phenolic content of 2,4-D-treated leaves

On the expectation that about 20% of the 2,4-D fed to strawberry leaves would be completely degraded to 2,4-dichlorophenol, an experiment was carried out to measure the increase in phenolic content of 2,4-D-treated strawberry leaves.

Phenols were extracted from batches of five leaves by maceration in 60 ml. water containing 0·7 g. sodium hydroxide. After hydrolysis under reflux for 30 min., 7 g. anhydrous sodium sulphate and 15 ml. 2N sulphuric acid were added and the mixture steam distilled until 30 ml. of distillate had been collected. Phenols were estimated colorimetrically on 10 ml. aliquots, using 5 ml. Folin Dennis reagent and 10 ml. 5% sodium carbonate. The colour was allowed to develop for 2 hr. at 25° C. and was then measured on a spectrophotometer at 700 mμ, using a 4 cm. cell. A calibration curve was prepared from a series of standard solutions of 2,4-dichlorophenol.

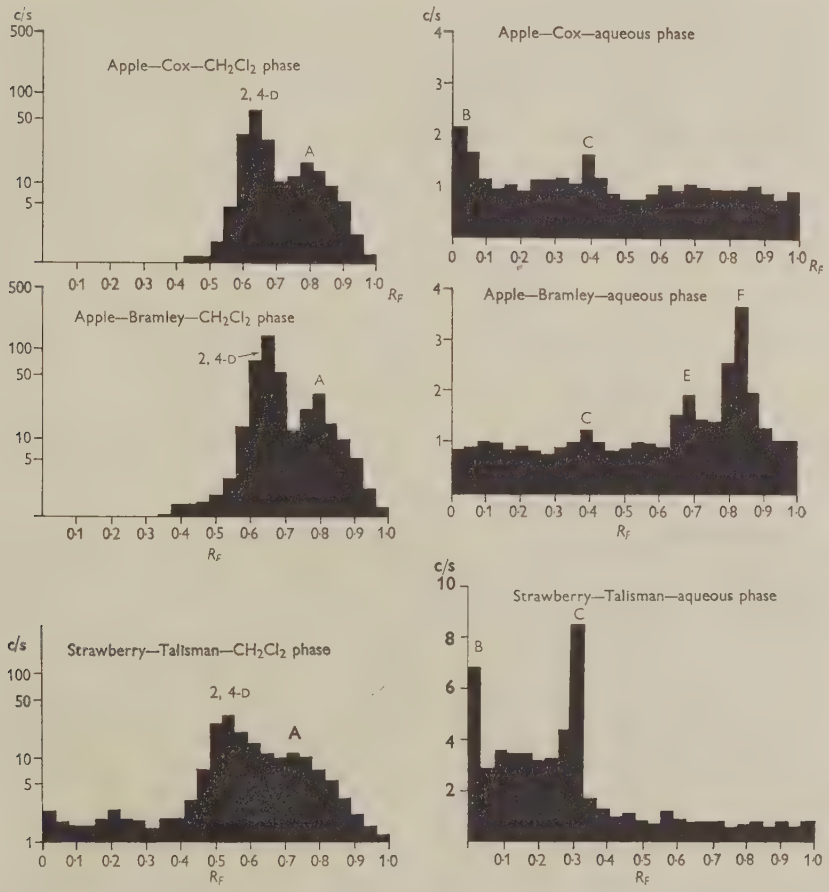
Twenty leaves of Talisman strawberry were allowed to take up a total of 3400 μg. 2,4-D from a 100 p.p.m. solution and were then transferred to jars of water. After 72 hr. 870 μg of phenolic substances, estimated as 2,4-dichlorophenol, were recovered in the 30 ml. distillates from these leaves. Twenty untreated leaves left for the same time in water yielded 90 μg. The increase of 780 μg. equivalents of 2,4-dichlorophenol in the treated leaves corresponds to the complete degradation of 23% of the 2,4-D, a figure in close agreement with that expected from studies of demethylation. Attempts to recover 2,4-dichlorophenol from 2,4-D-treated leaves without prior hydrolysis were unsuccessful.

Products of 2,4-D metabolism in apple and strawberry

Leaves of Cox and Bramley apple and Talisman strawberry, which had been allowed to metabolize carboxyl-labelled 2,4-D in darkness for 1 day were extracted with dilute ammonia and the extracts subsequently partitioned between water and

methylene chloride. The two phases, after concentration, were chromatogrammed in *iso*-propanol:water:ammonia (10:1:1) and the chromatograms scanned for radioactivity (Text-fig. 2).

In all three plants the radioactivity in the methylene chloride phase was due chiefly to unmetabolized 2,4-D, but 15–20% was present as a compound of higher



Text-fig. 2. Histograms showing distribution of radioactivity on chromatograms of aqueous and methylene chloride phases of extracts of apple and strawberry leaves which had metabolized carboxyl-labelled 2,4-D.

R_F value. This compound, which is non-acidic and shows auxin activity, is almost certainly the same as compound A described from red and black currant (Luckwill & Lloyd-Jones, 1960). The two water-soluble metabolic products in Cox and

Talisman leaves appear identical with compounds B and C in the red currant. In Bramley, a small quantity of C was found, but most of the radioactivity in the water phase was present as two compounds of much higher R_F value (E and F, Text-fig. 2), both of which showed some auxin activity in the oat mesocotyl test (Nitsch & Nitsch, 1956).

Decarboxylation of other phenoxy-acids by apple and strawberry

Leaves of apple (var. Cox's Orange Pippin) and strawberry (var. Talisman) were fed with 20 p.p.m. aqueous solutions of five different carboxyl-labelled phenoxy-acids. Table 3 shows the percentage of the carboxyl carbon recovered as carbon dioxide over a period of 20 hr. Of the five acids used, 2-CPA was the only one that was not decarboxylated to any appreciable extent.

Table 3. *Decarboxylation of five different substituted phenoxy-acids by leaves of apple and strawberry over a period of 20 hr. in darkness at 25° C.*

	Apple—Cox's Orange Pippin		Strawberry—Talisman	
Compound	Uptake (μg.)	% decarb-oxylated	Uptake (μg.)	% decarb-oxylated
2,4-D	46·8	24	68·2	25
2,4,5-T	16·4	34	74·8	44
2-CPA	25·8	1	66·2	1
4-CPA	30·2	60	59·2	24
MCPA	33·0	41	69·6	14

Factors influencing rate of decarboxylation

When carboxyl-labelled 2,4-D was introduced into a detached leaf of strawberry, red currant or resistant variety of apple the maximum rate of decarboxylation was reached about 5 hr. after the end of the uptake period. Afterwards the rate fell rapidly over a period of 40–50 hr. Since the respiration rate also was falling over this period (Text-fig. 1), due to progressive exhaustion of carbohydrate reserves, it seemed possible that decarboxylation and respiration might be linked.

In the experiment illustrated in Text-fig. 3 two leaves of Talisman strawberry containing 103 μg. carboxyl-labelled 2,4-D were placed in the dark and respiration and decarboxylation were followed over a period of 67 hr. The leaves were then removed from the respiration chamber and left in the light for 4 hr., after which they were returned to the chamber and the observations continued. As a result of the light treatment, during which more carbohydrate was synthesized, the respiration rate increased 5 times, whilst the rate of decarboxylation was approximately doubled.

A similar experiment with Talisman leaves is illustrated in Text-fig. 4. Here the leaves were fed initially with 62 μg. labelled 2,4-D and after 44 hr. in the dark, when the rate of decarboxylation had fallen to a low level, a further 60 μg. 2,4-D were fed into the leaves in light over a period of 3 hr. Following this treatment there was a 30-fold increase in the rate of decarboxylation. This experiment shows that the detached leaf, even after 40 hr. in darkness, is still capable of actively metabolizing

2,4-D and suggests that the rate of decarboxylation may be a function of the amount of 2,4-D present.

Confirmation of this was obtained by feeding strawberry leaves with varying amounts of labelled 2,4-D and measuring the mean rate of decarboxylation over the

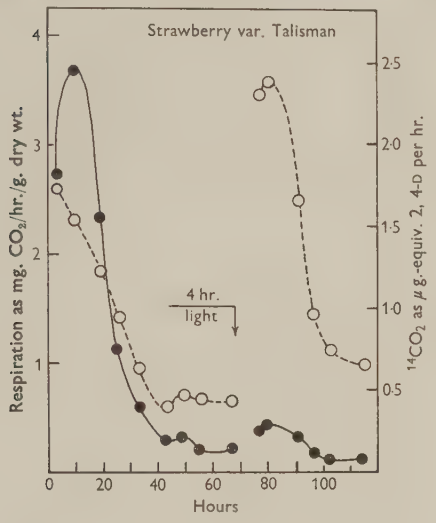


Fig. 3

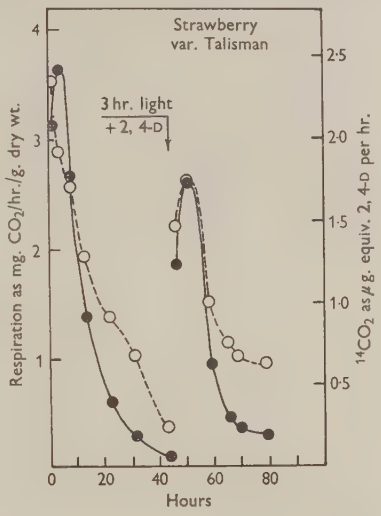
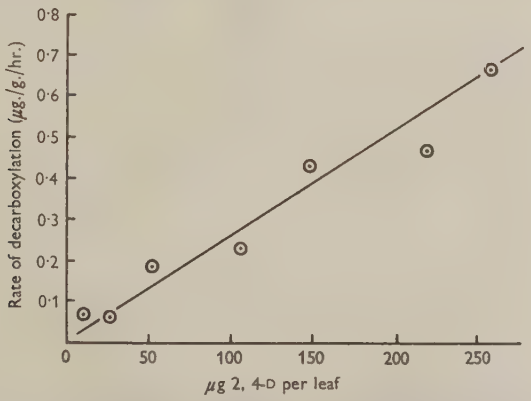


Fig. 4

Text-fig. 3. Respiration rate (broken line) and decarboxylation rate (solid line) of strawberry leaf treated with carboxyl-labelled 2,4-D (see text for details).

Text-fig. 4. Effect of second treatment with carboxyl-labelled 2,4-D on decarboxylation rate (solid line) and respiration rate (broken line) of strawberry leaves (see text for details).



Text-fig. 5. Relation between initial amount of carboxyl-labelled 2,4-D fed to strawberry leaves and the mean rate of decarboxylation over the first 40 hr. after treatment.

first 40 hr. The results, illustrated in Text-fig. 5, indicate a direct proportionality between the initial dose of 2,4-D and the rate at which it is broken down.

On the assumption that the end-product of side chain degradation is 2,4-dichlorophenol, the possibility that the accumulation of this compound in the leaf might have a depressing effect on metabolism was investigated. A leaf of Talisman was fed with 38 μg. 2,4-dichlorophenol from a 20 p.p.m. solution and subsequently with 30 μg. carboxyl-labelled 2,4-D. A control leaf received 30 μg. 2,4-D without pretreatment with phenol. The presence of 2,4-dichlorophenol in the leaf was found to have no effect on either the rate of decarboxylation of 2,4-D or the rate of respiration.

In a further experiment with leaves of the strawberry variety Talisman in which leaves of different ages were fed with carboxyl-labelled 2,4-D it was found that young leaves were able to decarboxylate the side chain at only about half the rate of fully grown or senescent leaves (Table 4).

TABLE 4. *Effect of age of leaf on rate of decarboxylation of 2,4-D by strawberry var. Talisman*

Condition of leaf	Fresh weight (g.)	2,4-D uptake (μg.)	Respiration rate (mg./hr./g.)	% 2,4-D decarboxylated in 20 hr.
Unfolding	0·90	18·0	0·43	16
Half-grown	1·45	57·8	0·46	16
Full-grown	2·50	71·2	0·39	30
Senescent	2·41	60·8	0·44	28

TABLE 5. *Decarboxylation of 2,4-D by detached leaves and shoots of miscellaneous species*

Species	Fresh weight (g.)	2,4-D uptake (μg.)	% 2,4-D decarboxylated in 21 hr.
Resistant			
Matricaria suaveolens	5·69	47·4	3·6
Agropyron repens	2·99	50·6	0·9
Pteridium aquilinum	2·05	21·6	7·6
Aegopodium podagraria	3·30	62·6	1·4
Agrostis stolonifera	2·89	60·4	0·7
Rubus fructicosus	3·12	45·8	3·4
Achillea millefolium	2·39	15·8	0·1
Crataegus oxycantha	1·44	25·0	0
Syringa vulgaris	3·65	18·2	22·4
Intermediate			
Veronica persica	5·55	34·4	1·0
Senecio vulgaris	4·23	49·4	7·7
Potentilla reptans	2·82	40·0	0·4
Susceptible			
Calystegia sepium	1·89	44·4	0·4
Prunus cerasus F/12/1	2·97	47·0	0·4
Plum—Severn Cross	2·25	26·8	1·4
Pear—Conference	2·27	35·0	0·7
Rose—Virgo	5·37	60·5	1·9

Decarboxylation of 2,4-D *by other species*

In view of the results obtained with cultivated varieties of currants, apples and strawberries a wider range of species, showing varying degrees of resistance to 2,4-D were tested for their ability to decarboxylate the side chain of this acid. Carboxyl-labelled 2,4-D from a 20 p.p.m. solution was fed into detached leaves or shoots of the species listed in Table 5, and the production of radioactive carbon dioxide followed over a period of 21 hr., the leaves being kept in darkness at a temperature of 25° C.

All the 2,4-D-susceptible species tested showed low rates of decarboxylation, as did most of the species of intermediate and high resistance. The only other plant found with the ability to decarboxylate 2,4-D at a rate comparable with that of apple and strawberry was an unidentified variety of garden lilac (*Syringa vulgaris*). A test, in which cut shoots were dipped in 2,4-D solutions of varying strength, confirmed that this species was highly resistant.

Discussion

These studies of 2,4-D metabolism in the leaves of apple and strawberry confirm and extend the results previously published for red and black currants. At least in these groups of plants it is clear that resistance to 2,4-D is closely associated with ability to oxidize the side chain of the acid to carbon dioxide. On the other hand, it appears from the data in Table 5 that this relationship is not of general occurrence, and that other groups of plants must have other mechanisms of resistance. This conclusion is supported by the work of Weintraub, Reinhart & Scherff (1956) who carried out tests with twelve varieties of maize of varying sensitivity to 2,4-D and reported that they could find no relationship between growth response and decarboxylation.

In the present series of experiments the absence of decarboxylation in the resistant Hawthorn (*Crataegus oxycantha*), belonging to the same family as apple and strawberry, is particularly significant, as is also its occurrence in the non-related red currant and garden lilac. Ability to decarboxylate phenoxy-acids presumably depends on the presence of a specific enzyme system, which seems to occur in isolated genera of a number of different families. Some evidence that this ability is an inherited character was obtained for apples.

Breakdown of 2,4-D by degradation of the side chain will not necessarily confer resistance unless the plant also possesses a mechanism for the detoxication of the 2,4-dichlorophenol which, there is good reason to suppose, is the end product of the reaction. Apples, strawberries and currants all appear to possess such a mechanism, as relatively large quantities of 2,4-dichlorophenol fed to leaves through the transpiration stream produce no phytotoxic effects; neither is the respiration rate of the leaf affected by this treatment. The fact that phenolic compounds can be recovered from 2,4-D-treated leaves only after hydrolysis suggests that glycoside formation may be the mechanism of detoxication. Miller (1941*a*, *b*) has shown that a number of toxic chemicals, including 2-chlorophenol, may be inactivated in this way when fed into tomato roots or *Gladiolus* corms.

In strawberries, as in red currants, MCPA, 4-CPA and 2,4,5-T proved much more damaging than 2,4-D even though they were decarboxylated at approximately the same rate. The first signs of damage, which appeared approximately 1 week after treatment, was a characteristic yellowing of the foliage. This was particularly marked in plants treated with 2,4,5-T. Osborne (1958) has observed a similar yellowing of the foliage in certain tropical species after treatment with 2,4,5-T butyl ester. The fact that this type of damage is not observed in black currant, a species which is unable to decarboxylate 2,4,5-T, strongly suggests that the yellowing and subsequent death of the leaf is due to the formation of 2,4,5-trichlorophenol which, for some reason, is not detoxicated as readily as 2,4-dichlorophenol.

The other metabolic products formed from 2,4-D in the leaves of apple and strawberry appear to be similar to those already reported for currants and discussed in the first paper of this series.

The authors gratefully acknowledge the help of Mr R. G. Powell of the A.R.C. Unit of Experimental Agronomy, Oxford, who supplied the labelled phenoxy-acids other than 2,4-D. They also thank Mr P. Weaver, Miss M. R. Manning and Miss J. Parsons for technical assistance.

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EXPLANATION OF PLATE 10

Fig. 1. Maiden trees of Bramley's Seedling (left) and Cox's Orange Pippin (right) 6 weeks after spraying with various concentrations of 2,4-D. A = controls; B = 30 p.p.m.; C = 120 p.p.m.; D = 500 p.p.m.

Fig. 2. The reaction of strawberry var. Talisman to sprays of five different phenoxy-acids at concentrations of 250, 500, 1000 and 2000 p.p.m. Controls (left) were sprayed with wetter only. Photographed 10 weeks after spraying.

(*Received 27 January* 1960)

Fig. 1

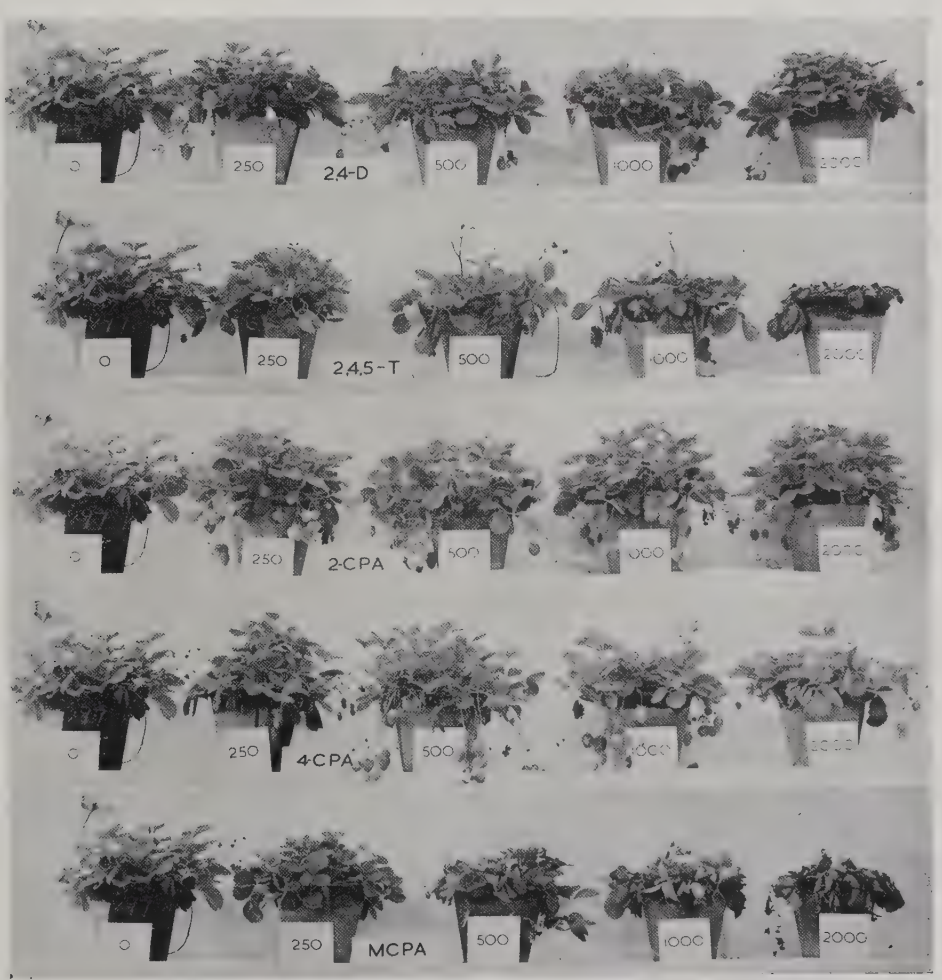

Fig. 2

LUCKWILL AND LLOYD-JONES—*Metabolism of plant growth regulators. II*

(*Facing p.* 636)

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AN ANALYSIS OF COMPETITION BETWEEN BARLEY AND WHITE PERSICARIA

II. FACTORS DETERMINING THE COURSE OF COMPETITION*

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(With 7 Text-figures)

White persicaria grown with barley had a lower relative growth rate, a lower net assimilation rate, a higher leaf-area ratio and a higher leaf-area : leaf-weight ratio than when grown alone. These differences were accentuated with time and with increase in the density of the surrounding barley plants. That the responses were those of plants subjected to a high degree of shading was confirmed by growing spaced plants under differing degrees of shade and from measurements of the amount of light penetrating the mixed stands. However, they do not necessarily demonstrate that light was the environmental factor in shortest supply.

An attempt was made to distinguish the effect of competition between shoots from competition between the roots. Root competition appeared to reduce growth more than shoot competition although the influence of the latter increased with time. Increase in nutrient supply, which was varied by supplying, at weekly intervals, solutions of various dilutions, caused a proportionately greater increase in the growth of white persicaria than of barley in a mixed population.

These results suggest that restricted nutrient supply was the factor which first decreased the growth of white persicaria. This was a direct consequence of its smaller root system arising from a smaller embryo than that of barley. The divergence between the amount of growth of the two species with time led to the white persicaria plants becoming progressively more and more shaded, and ultimately caused a further reduction in the rate of expansion of the root system and of the leaves. Although restriction in light was certainly concerned, as shown by the type of plant produced, it seemed likely that mineral nutrients were the factors always in shortest supply.

The reduction in tiller number, but not in dry weight, of barley when grown with white persicaria was interpreted as a response to a lower internal concentration of nitrogen arising directly from the lower external concentration. Root extension did not appear to be significantly reduced.

In a previous paper (Aspinall & Milthorpe, 1959) the relationships between the growth of barley and of white persicaria (*Polygonum lapathifolium* L.) in pure and mixed populations were described. Persicaria was greatly restricted when associated with barley, whereas barley was little influenced by the presence of persicaria even at high densities. Also, mutual retardation of the growth of plants in pure populations occurred at much lower densities with barley than with persicaria. These differences in competitive ability arose directly from the greater size of barley plants

* The data presented in this paper were included in a thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy.

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at emergence, the relative growth rate of persicaria being as high or higher than that of barley when grown separately under widely spaced conditions.

Although related to the amount of growth, the effects observed must arise through local changes in one or more environmental factors such as light or nutrient supply. The relative importance of these factors and the growth responses which occur within competing systems are still obscure. An attempt was made during the present investigations to explore these aspects using data derived from the experiments previously described and from other experiments which are described in the appropriate sections.

The influence of competition on growth attributes (*Experiments* 1 *and* 2)

Two experiments were described in the first paper, barley and persicaria plants being grown separately or mixed over a range of densities. From the data presented therein, relative growth rates, net assimilation rates and leaf-area ratios were calculated and are presented here.

TABLE 1. *Relative growth rates, net assimilation rates, and leaf-area ratios of white persicaria and barley grown in pure and mixed populations*

(Exp. 1: mean values over the first three inter-harvest periods with standard errors of means in parentheses*)

Species	Relative growth rate (10^{-1} g./g./day) ($\pm$0·117)	Net assimilation rate (10^{-3} g./cm.^{2}/day) ($\pm$0·062)	Leaf-area ratio (10^2 cm.^{2}/g.) ($\pm$0·032)
Pure populations			
White persicaria	1·52	1·06	1·31
Barley	1·09	0·98	1·03
Mixed populations			
White persicaria	1·20	0·58	1·93
Barley	1·17	1·05	1·04

* The standard errors presented in Tables 1 and 3 are indicative rather than absolute. Complete independence of each derived value has been assumed although one set of original observations is common to each consecutive pair of derived values.

In Exp. 1 (cf. part I, Aspinall & Milthorpe, 1959, p. 158) the two species were grown separately and mixed in equal proportions with 4, 8, 16 and 32 plants per pot. Over the first 8 weeks of growth, which covered the period from emergence to maximum leaf area of barley, the relative growth rate of persicaria in pure populations was higher than that of barley (Table 1). This difference arose from a higher leaf-area ratio, the net assimilation rates being similar. When associated with barley, however, the net assimilation rate of persicaria was greatly reduced and, despite an increase in the leaf-area ratio, the relative growth rate was considerably less. Barley, however, was not influenced by association with persicaria.

There were marked differences in the changes with time of these growth attributes (Fig. 1). The net assimilation rate of persicaria when grown alone increased throughout with time, whereas it progressively decreased when this species was grown with barley. There was a progressive decrease with time in the net assimila-

tion rate of barley grown in either type of association. Marked differences between the leaf-area ratios of persicaria plants grown alone and with barley contrasted with the absence of any effect of association on the leaf-area ratio of barley.

The lack of any influence of persicaria on changes in these growth attributes of barley confirm that the latter did not experience any 'competition' stress. The marked changes induced in persicaria by barley were all of a nature which suggested that reduction in light intensity was the operative factor. The reduction in growth rate with increase in density which was shown by barley when grown in pure populations (cf. part I, fig. 2) was accompanied by a decrease in the net assimilation

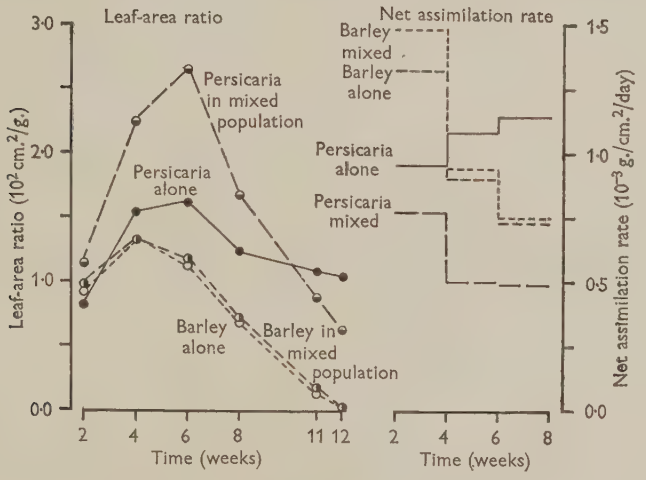

Fig. 1. The relationships of the leaf-area ratio and the net assimilation rate with time (Exp. 1).

rate without any change in the leaf-area ratio (Aspinall, 1957). This is but a partial response to increased shading and suggests that some factor other than light was also operating.

In Exp. 2 (cf. part I, p. 160) growth attributes were compared of single persicaria plants when surrounded by barley at different densities. Similar comparisons were made for single barley plants surrounded by persicaria. As in Exp. 1, the leaf-area ratio was higher but the net assimilation rate and relative growth rate were lower in persicaria than in barley (Fig. 2). The leaf-area ratio of persicaria followed a similar trend with time to that found in Exp. 1, but no pronounced change with time was seen in barley. In both species, however, the net assimilation rates and relative growth rates fell with time.

Increase in the density of barley plants surrounding a central plant of persicaria greatly decreased the relative growth rate of the latter (Fig. 3). There was no such effect with a barley plant surrounded by persicaria at equivalent densities except

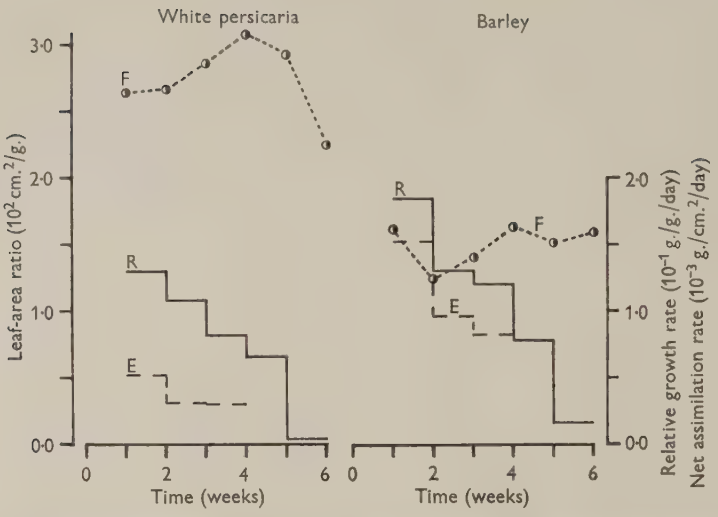

Fig. 2. The relationships with time of the leaf-area ratio (F), relative growth rate (R) and net assimilation rate (E) of white persicaria and of barley when grown as central plants (Exp. 2).

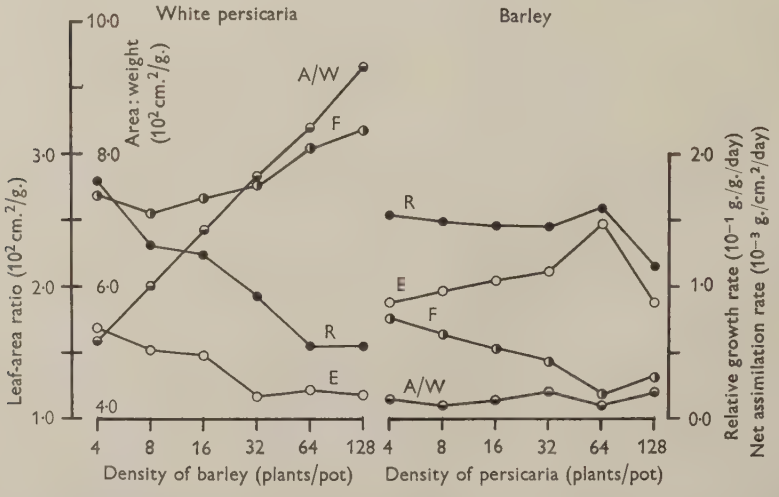

Fig. 3. The relationships of growth attributes of a central plant of white persicaria with density of surrounding barley plants and of barley with density of surrounding white persicaria plants. R, relative growth rate; E, net assimilation rate; F, leaf-area ratio; A/W, ratio of leaf-area to leaf weight. (Density is plotted on a logarithmic scale.)

at the highest density. Over the range of 4–64 persicaria plants per pot, the net assimilation rate of barley *increased* and its leaf-area ratio *decreased*, resulting in a constant relative growth rate. With barley surrounded by 128 persicaria plants and with persicaria surrounded by barley at all densities, the lowering of the relative growth rate was accompanied by a *decrease* in the net assimilation rate and an *increase* in the leaf-area ratio. As in Exp. 1, the growth responses of plants subjected to competition suggested that the operative factor was reduction in the light supply.

The leaf-area ratio (leaf area:total plant weight) may be resolved into two components—the leaf-area:leaf-weight ratio and the leaf-weight:total plant-weight ratio. The increase in the leaf-area ratio of persicaria with increasing competition was accounted for by an increase in the ratio of leaf area of leaf weight (Fig. 3), i.e. a similar proportion of assimilate was diverted to leaf production at all densities but at the higher densities each unit of this was used in the production of larger thinner leaves. On the other hand, the changes in the leaf-area ratio of barley were reflected by changes in the proportion of leaf weight to the total plant weight, the ratio of leaf area to leaf weight remaining constant. The increase in the leaf-area ratio being wholly through increase in the ratio of leaf area to leaf weight is a typical response of a dicotyledonous plant to shading (Blackman, 1956; Blackman & Wilson, 1954). Gramineous plants respond similarly to shading but the ratio of leaf weight to total plant weight is also increased. In barley, leaves formed a decreasing proportion by weight of the total plant as the density of the surrounding persicaria was increased. This is a response which occurs with increase in light supply or decrease in nutrient supply or increase in age. It may be concluded therefore that persicaria did not significantly influence the light supply to the central barley plant but it may well have reduced the supply of nutrients.

Reduction in light intensity resulting from increase in density (Experiments 1 and 2)

An attempt was made to measure directly the light environment in the two competition experiments (Exps. 1 and 2). Light intensities were measured at soil level by means of a small photocell and expressed relative to that simultaneously reaching the top of the plants. Records were made at all densities at intervals throughout the experiment, but only on days with a full cloud cover and within one hour of noon. The readings presented (Fig. 4) were those at soil level at the centre of the pot. The presence of shields prevented lateral illumination, the intensity varying only slightly across the surface of the pot at any one time in a uniformly distributed population of plants.

In Exp. 1, barley intercepted more light than persicaria when grown at the same density and for the same length of time (Fig. 4). This difference was maintained for about 4 weeks, by which time the highest density of persicaria intercepted as much light as the highest density of barley, but differences between the two species at lower densities were not eliminated until much further growth had been made. The only records of light intensity presented from Exp. 2 are those from the base

of the persicaria surrounded by barley at varying densities. Considerable interception of light occurred early in the experiment; by the first harvest, 40% was intercepted at the lowest density and about 70% at the highest density. These differences were reduced as the plants grew and the proportion of light intercepted increased; at 4 weeks after planting, 91 and 98% was intercepted at the lowest and highest densities respectively.

These measurements were made at the base of the plant only and give little more than an approximation of relative differences in the light environment. The appropriate measurement for correlation with growth responses would have been the integrated mean of measurements made at different heights adjacent to the central persicaria plants (in Exp. 2). Published observations (Black, 1958; Rademacher, 1940) indicated that the proportion intercepted diminishes gradually with height above the surface. An approximate estimate of the mean incident light may be

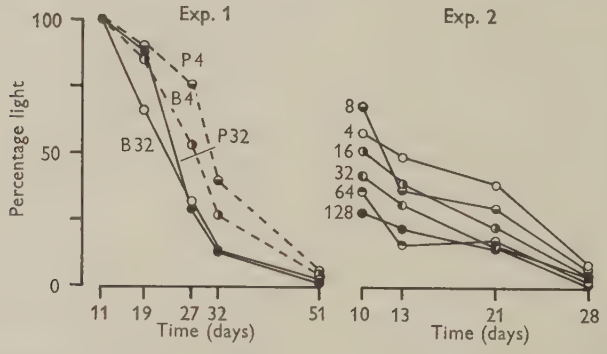

Fig. 4. The proportion of light penetrating to the soil level expressed as a percentage of that reaching the top of the plants. Changes with time are shown for pure populations of white persicaria (P) and barley (B) at densities of 4 and 32 plants per pot (Exp. 1) and for a single plant of white persicaria surrounded by barley at densities of 4, 8, 16, 32, 64 and 128 plants per pot (Exp. 2). (The time scales are comparable with those used previously; that of Exp. 1 commences at emergence and that of Exp. 2, 7 days later.)

obtained by extrapolation from the functions obtained by Rademacher for a short-strawed variety of oat. At the 21st day, the persicaria plants were about one-quarter of the height of the surrounding barley. Reduction in growth was then evident at densities of 8 and greater. At a density of 8, the light incident on the persicaria was about 30% at the base and 40% at the top of the plant, giving, say, a mean intensity of 35% full glasshouse light. At a density of 16, the incident light would be expected to vary between 23% and about 35%, giving a mean incident intensity of the order of 25–30% glasshouse light, and with 128 plants the mean incident intensity was calculated as 15–20%. These are amounts of shading at which some reduction in growth would be expected (p. 644); that is, at the time when reduction in the growth of persicaria was first detectable, the intensity of light within the crop was such as to make it a possible causal factor.

The relative growth rates of spaced plants (*Experiment* 3).

The experiments already described suggested that persicaria had a higher inherent growth rate than barley because it had a higher leaf-area ratio. This finding was explored further in an experiment not previously reported (Exp. 3) by measuring the growth attributes of single plants of each species during the early stages of growth. The experimental conditions were similar to those described previously (I, p. 157) except that 3 l. tins were used in place of 10 in. pots. An excess of seeds were planted and thinned to one plant per in. on emergence. When the majority of persicaria plants had one and barley plants two expanding leaves the plants were grouped in pairs, the members of each pair having an equal area of leaf surface. Five pairs of each species were taken, one plant of each pair being harvested immediately and the other 7 days later. The experiment was repeated five times during the summer of 1956.

TABLE 2. *Relative growth rates, net assimilation rates, leaf-area ratios, and relative leaf area growth rates of white persicaria and barley, together with the mean daily radiation, over 7-day periods during the early stages of growth*

(Significant differences between species, $P<0.05$, are shown by entries in heavy type.)

Date of initial harvest	Mean radiation (cal./cm.²/day)	Species	Relative growth rate (10^{-1} g./g./day)	Net assimilation rate (10^{-3} g./cm.²/day)	Leaf: area ratio (10^{2} cm.²/g.)	Relative leaf area growth rate (10^{-1} cm.²/cm.²/day)
7 May	232	White persicaria	**2·73**	**1·42**	**1·94**	**2·47**
		Barley	**1·48**	**0·93**	**1·59**	**1·50**
22 May	337	White persicaria	**3·20**	**1·51**	**2·14**	**2·98**
		Barley	**1·97**	**1·20**	**1·65**	**1·95**
6 June	172	White persicaria	2·01	0·81	**2·51**	1·67
		Barley	1·74	0·84	**2·08**	1·65
16 July	206	White persicaria	**1·87**	0·70	**2·68**	**1·82**
		Barley	**1·39**	0·70	**1·99**	**1·14**
24 August	145	White persicaria	1·39	0·74	**1·91**	1·17
		Barley	1·29	0·81	**1·63**	1·58

Persicaria always had a higher relative growth rate than barley, associated with a higher leaf-area ratio and a higher relative rate of leaf expansion (Table 2). Although this species difference was shown on all occasions, differences in relative growth rate within each species between occasions were more closely related to net assimilation rate than leaf-area ratio. Both relative growth rates and net assimilation rates of persicaria were fairly closely related to the radiation received in the glasshouse during the experimental period, but the relationships were far less close with barley. Hence, although persicaria may be expected to grow more rapidly than barley in any given local environment it also responds more markedly to changes in environmental factors such as radiation.

The influence of shading on the growth of persicaria and barley (Experiment 4)

The influence of shading on the growth of the two species was investigated by the technique of Blackman and co-workers (cf. Blackman & Wilson, 1951*a*). Spaced plants (1 plant per 36 in.^{2}) were grown in small field plots, with guard rows, under full radiation, 48% radiation (under zinc gauze shades) and 40% sunlight (under butter muslin shades). On six occasions during the growing period samples were taken for determinations of dry weight and leaf area; data from the first four harvests are presented in Table 3.

TABLE 3. *The effect of shading on the relative growth rates, net assimilation rates and leaf-area ratios of spaced plants of white persicaria and barley during their growth*

Species	Light intensity (% full sunlight)	Growth period (days from sowing) 44–59	59–68	68–81
	Relative growth rate (10^{-1} g./g./day) ($\pm$0·196)			
White persicaria	100	2·04	1·20	0·42
	48	2·10	1·00	0·47
	40	1·30	1·10	0·54
Barley	100	0·96	0·85	0·21
	48	0·91	0·86	0·31
	40	0·82	0·60	0·38
	Net assimilation rate (10^{-3} g./cm.^{2}/day) ($\pm$0·170)			
White persicaria	100	1·13	0·93	0·60
	48	0·83	0·52	0·49
	40	0·51	0·53	0·49
Barley	100	0·83	1·35	0·67
	48	0·60	1·00	0·74
	40	0·47	0·59	0·75
	Leaf-area ratio (10^2 cm.^{2}/g.) ($\pm$0·094)			
White persicaria	100	1·99	1·38	0·79
	48	2·51	2·05	1·08
	40	2·68	2·16	1·28
Barley	100	1·34	0·69	0·34
	48	1·72	0·94	0·49
	40	1·87	1·13	0·57

Over the period studied, there was no appreciable effect of shading on the relative growth rate of either species; that of persicaria was higher than that of barley throughout. With both species, however, shading decreased the net assimilation rate and increased the leaf-area ratio. Both these species therefore showed responses to shading similar to those of species studied by Blackman and colleagues (Blackman, 1956). Also, the relative growth rates were little affected unless the light intensity was decreased to less than 40–45% of full summer daylight. At lower intensities the decreased net assimilation rate was not fully compensated by increase in leaf-area

ratio of either species, and so relative growth rate was decreased. Assuming a linear relationship between net assimilation rate and log. light intensity (Blackman & Wilson, 1951*a*) the compensation points of both species appear to be between 10 and 15% full daylight.

The relative importance of competition between roots and between shoots (*Experiment* 5)

The relative importance of light and nutrient supply in reducing growth when plants competed with each other was investigated in another way by providing different spatial arrangements for the roots and shoots and assessing the influence of 'root association' (i.e. nutrient supply) and 'shoot association' (i.e. light supply)

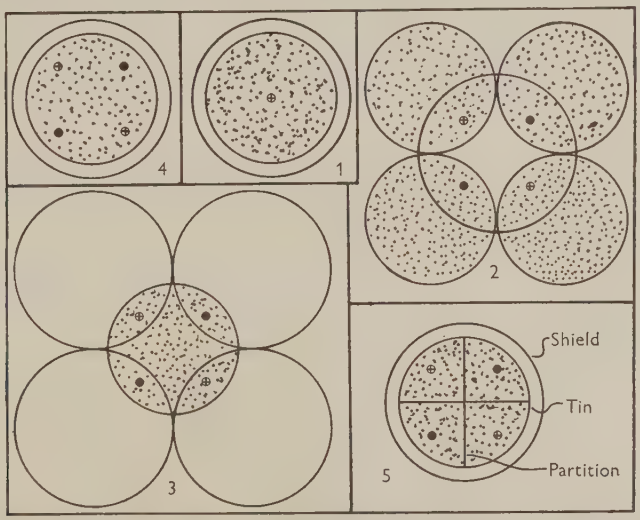

Fig. 5. Diagrammatic representation of treatments used in Exp. 5. Large stippled circle indicates sand in tin, large white circle indicates radiation shield, small white circle with cross indicates one subordinate plant and small black circle indicates eight dominant plants. The numbers refer to the treatment (cf. this page).

on subsequent growth. Since the completion of these experiments, Donald (1958) has published results obtained by a similar though more elegant technique.

Root and shoot competition were controlled by the manipulation of two experimental units: (i) the root container which consisted of a 3 l. tin with provision for drainage, coated with Bitumastic paint, filled with sand and sunk to its rim in a sand plunge-bed; and (ii) the shoot container which provided a shield from lateral radiation and was made from a tin of the same diameter as the root container. The treatments (Fig. 5) were as follow:

(1) *No competition.* One plant of the subordinate species was grown per tin and provided with a radiation shield.

(2) *Shoots only in competition.* The plants were grown in four separate tins, two tins each with one subordinate plant and two tins each with eight dominant plants. All the shoots were constrained to grow within the one centrally placed radiation shield.

(3) *Roots only in competition.* Two groups of one subordinate plant and two groups of eight dominant plants were grown in the one tin but the shoots were separated so that those of each group grew in a separate shield.

(4) *Both roots and shoots in competition.* This was similar to treatment 3 except that all shoots grew together within the one radiation shield.

(5) Shoots only in competition but roots restricted. The plants were grown as in treatment 4, except that each group was planted in one of four equal waterproof sections. This treatment was included to provide an estimate of the amount of growth which would have been expected in treatment 3 if roots did not penetrate beyond their respective quarter-cylinder and of the differences in growth between treatments 2 and 4 which were attributable to differences in the available volume of nutrient solution.

TABLE 4. *Growth* ($\log_e$ *dry weight per plant in* 10^{-1} *g.) at two harvests of white persicaria and barley as subordinate and as dominant plants with differing association between roots and shoots*

		White persicaria at (days from emergence)		Barley at (days from emergence)	
Treatment	Components competing	34	56	34	56
	Species as subordinate plants	(±0·227)			
1	None	2·28	5·29	4·05	5·19
2	Shoots only	2·34	4·27	3·32	4·58
3	Roots only	0·88	1·94	3·22	3·83
4	Shoots and roots	1·01	1·20	3·35	3·84
5	Shoots (roots restricted)	2·05	3·39	3·17	4·13
	Species as dominant plants	(±0·121)			
1	None (single plant)	2·28	5·29	4·05	5·19
2	Shoots only	2·14	3·51	2·49	3·06
3	Roots only	1·44	2·46	2·15	2·59
4	Shoots and roots	1·38	2·28	2·32	2·53
5	Shoots (roots restricted)	1·20	2·19	1·70	2·13

There were two populations, i.e. with barley or with persicaria as the subordinate species. Nutrient solution of the same composition as used previously was applied to each root container weekly. Harvests were made at 34 and 56 days after emergence when the dry weights of roots, stems and leaves were determined separately. There were three replicates.

Competition between the shoots in treatment 2 depended upon bringing the shoots from separate tins together. Although the plants were sown in adjacent corners of the tins this was difficult to achieve at an early stage of growth because of the large central space between the tins. The metal shields could not be placed around the plants until 11 days after emergence. They were adjusted in height later in the experiment by adding a second and then a third shield. The adjustment of

these shields, each of which was 9 in. high, was not as sensitive or as frequent as in the previous experiments because they could not fit around the lower tins.

The dry weights were transformed to logarithms for analysis. Where persicaria was the subordinate species (one plant of persicaria surrounded by eight plants of barley) and competition effects were attributable to the barley, there was much greater reduction in growth from association between roots than between shoots (Table 4). By the second harvest, competition between shoots was more evident, but its effect was still less than that between roots. With barley as the subordinate species, similar responses were found, but these were of a much smaller magnitude. When persicaria was dominant in numbers (eight plants to one plant barley) with responses mainly from intraspecific competition plus a small contribution from the subordinate plant of barley, reduction in growth arising from association between shoots was greater than when it was subordinate, but association between roots was still the chief factor inducing retardation of growth. Barley was influenced more by association with plants of its own species than with plants of persicaria; association between roots again had a larger effect than between shoots. Only with

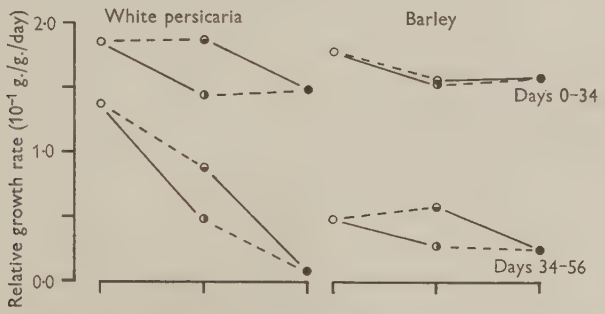

Fig. 6. Interaction diagrams showing the influence of root and shoot competition on the relative growth rates of subordinate plants of white persicaria and of barley over the periods 0–34 days and 34–56 days from emergence. ○, indicate no competition; ◑, root competition only; ◒, shoot competition only; ●, both root and shoot competition. Continuous and broken lines show the influence of introducing root competition and shoot competition respectively.

persicaria at the last harvest was there an additive effect of association; then the reduction in growth when shoots and roots were associated was as great as the sum of each acting separately.

Mean relative growth rates over each of the two periods were calculated assuming weights at emergence of 13·5 mg. for barley and 1·8 mg. for persicaria (Fig. 6). These show that the greater dry weights of barley than of persicaria at the first harvest arose mainly from the differences in initial weight. During the second period the relative growth rate of barley was much lower than that of persicaria, presumably because ear differentiation and emergence were then occurring. This figure also shows more clearly than Table 4 the important influence of root

competition in both species, and the fact that shoot competition was evident with persicaria only—and then only after this species has been subjected to root competition for some time.

The importance of the root environment in inducing the effects arising from competition was also shown by the comparison of treatment 5 (roots of the two species separated) with treatment 4 (roots intermingling in the same volume of sand) and with treatment 2 (roots separated in four times the volume of sand) (Table 4). Growth was always greater in treatment 2 than in treatment 5, which suggested that it was restricted by nutrient supply as controlled by the available volume of nutrient solution even when the shoots were in competition. When persicaria was subordinate (one plant per quarter-cylinder) growth was less in treatment 4 than in treatment 5. This suggests that a smaller 'effective' volume of sand was available for persicaria in treatment 4 than in treatment 5, i.e. that roots of the dominant barley invading the root zone of the persicaria depleted the potential nutrient supply. No such response was found with the subordinate barley plants, which suggests that root extension by one barley plant was effectively comparable with that from the border of eight persicaria plants. When persicaria was dominant, no differences were found between treatments 4 and 5. Growth of the dominant barley plants was greater in treatment 4 than in treatment 5 where extension of roots into a larger 'biologically' available volume of nutrient solution was prevented by the physical barriers imposed.

In this experiment, association between roots caused greater reduction in growth than did association between shoots. The experimental procedure was biased in this direction because top competition could not be imposed during the first 11 days of growth. Nevertheless, root association was extremely important in reducing growth and probably operated earlier than did association between tops. The component tissues were influenced more or less proportionately. There were no marked differences in the ratio of leaf weight to the total plant weight, all possible trends being masked by the larger changes with time. With persicaria as the subordinate species the ratio of leaf weight to plant weight tended to be larger when there was competition between either roots or shoots alone but not when both were present together.

The influence of concentration of the nutrient solution (*Experiment* 6)

The effect of concentration of the nutrient solution on the differential growth of the two species when associated was explored. Plants were grown in sand in 10 in. plant pots as before, sixteen of each species being spaced alternately in each pot. Nutrient solution was applied weekly at the concentration used previously (part I, p. 157) or at 60, 40 and 20% of that concentration, sufficient being used at each application to displace all the solution in the pot. Care was taken to ensure that there was no loss by drainage between the applications. Lateral illumination was prevented after 8 days from emergence. All plants of the six replications were harvested 56 days after emergence.

Reduction of the nutrient supply through decreasing the concentration of the

nutrient solution to 60% of that previously used did not influence the growth of barley (Fig. 7), but further reduction did lead to less growth. However, the yield of persicaria was linearly related to the concentration over the entire range explored. This caused a change in the relative amounts of growth of the two species, the ratio by weight of persicaria to that of barley increasing with increase of concentration.

The changes in weight of both species were restricted almost entirely to the shoots; there was little difference between concentrations in weights of roots (Aspinall, 1957). With barley, the differences arose mainly from a greater production of tillers (one per plant at the lowest compared with three at the highest concentration) and of leaves but there was no difference in height (24·2 and 24·0 cm.). With persicaria, increase in nutrient concentration also increased branching (from 7 to 10·5 branches per plant) but this was delayed and occurred mainly from the upper nodes. The main responses were a large increase in the plant height (from 9·5 cm.

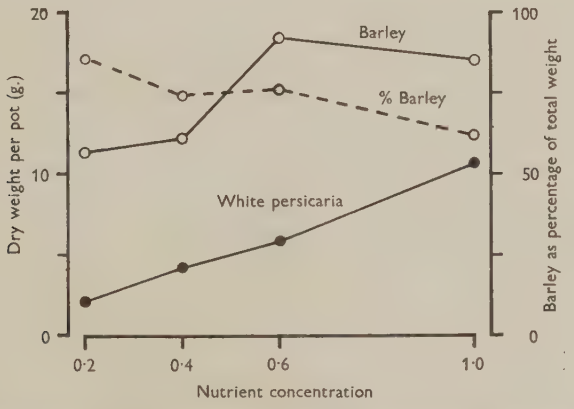

Fig. 7. Total yield (—) of white persicaria and of barley and proportion of barley (- - - -) when grown with various dilutions of the nutrient solution normally used.

at the lowest to 17·3 cm. at the highest concentration), and the production of more leaves on the main stem. These led to a difference in the vertical distribution of leaves of the two species, interception of light by persicaria being much increased by increase in concentration of the nutrient solution.

Discussion

Competition—the restriction in growth that arises from association with other plants—must result from a change in one or more factors of the local environment of a plant. Light and mineral nutrients are the two components most likely to be involved; water may also be concerned as a secondary factor in the field but not in the present experiments. Opinions regarding the relative importance of light and

nutrient supply range from 'competition begins as soon as the root system of one plant invades a feeding area of another, and usually takes place long before tops are developed sufficiently to exert serious competition for light' (Pavlychenko, 1940) to 'light is the factor which determines the ultimate yield of a community of plants of a particular species' (Donald, 1951). There have been few attempts, however, to verify these opinions of the lower and upper limits of the range of conditions found in competing systems or to explore experimentally the relative importance of light and of nutrient supply during the intermediate stages.

In the present experiments, growth of persicaria was considerably restricted by the presence of barley. It showed the characteristic responses of a plant subjected to shade (Blackman, 1956; Blackman & Wilson, 1951*a*): the relative growth rate and net assimilation rate were reduced, whereas the leaf-area ratio and the ratio of leaf area to leaf weight were increased. Reduction in the supply of nutrients would be expected to lead to a decrease in the leaf-area ratio (Blackman & Rutter, 1948; Blackman & Wilson, 1951*b*) and to have little or no effect on the net assimilation rate (Watson, 1952). Persicaria, when widely spaced and subjected to artificial shading, responded in a similar manner. Measurements of light within a stand of persicaria and barley suggested that the radiation incident on the former was reduced to intensities at which shading responses would be expected. These results all show that persicaria experienced and was no doubt influenced by low light intensities but do not necessarily demonstrate that light was the factor in shortest supply. Restricted nutrient supply must also be considered both as a primary factor and as an indirect consequence of the reduced light supply. One cannot agree entirely with Black (1958) who interpreted the responses found in a mixed association of subterranean clover of different initial size solely in relation to detailed measurements of light.

The experiment with varied spatial distributions of roots and shoots showed that competition between roots caused a greater decrease in growth than did competition between shoots, although the influence of shoot competition increased with time. Competition between both roots and shoots caused a greater decrease in the growth of persicaria than root competition alone but there was no such additive effect on the growth of barley when surrounded by persicaria. Similar results were obtained by Donald (1958) from an experiment in which the effects of shoot and root competition between two grasses were separated. Growth of the less successful competitor (*Phalaris tuberosa*) was reduced much more by association between roots than by association between shoots; growth of the successful competitor (*Lolium perenne*) was reduced only by association between the roots.

The much greater relative response of persicaria than of barley to increase in the concentration of the nutrient solution applied weekly (Exp. 6) also suggested that nutrient supply was involved. This experiment did not distinguish between two alternatives: (i) persicaria may require a higher external concentration than barley for maximum growth; or (ii) the effective supply from the roots of persicaria may have been less than from the roots of barley, because of the greater development of barley roots at any time, and, hence the higher the concentration of the frequently

renewed soil solution (or the more frequent its renewal) then the more would the species with the less-developed root system be favoured. This second explanation seems the more likely.

These experiments and that of Donald suggest that the decreased growth of the less successful of two associated species is at first due to restricted nutrient uptake, associated with a root system which is always smaller. As the roots expand the only space available is that already occupied by roots of the aggressor species or that from which some of the ions have diffused to be absorbed by the aggressor. This is likely to be true in the field as well as in pots because the roots can find soil solution of the original nutrient status only by extending downwards. Differences in plant density will influence only the length of time before the root systems intermingle but in all associations roots will interfere long before shoots of the aggressor begin to shade the less successful competitor. The species with the most extensive root system at the stage when competition begins is therefore the most likely to succeed. The advantage of a larger root system may be the result of greater embryo size, higher relative growth rate of the roots, or earlier germination. The advantage of a high initial relative growth rate will be soon lost if the species germinates later or has a smaller initial size than a slower-growing competitor, because the soil will be already occupied by its competitor in spite of its slower growth. The lack of success of persicaria is due to its smaller embryo size and its consequent smaller root system during the early stages of germination. The same effect possibly also contributes to the lack of success of small-seeded subterranean clover when grown with large-seeded varieties (Black, 1958) and of *Phalaris* in association with *Lolium* (Donald, 1958), although *Phalaris* possibly also has a lower relative root growth rate associated with less tillering.

The depression of growth by a reduced supply of nutrients soon leads to the shoots of the suppressed species being shaded by the leaves of the aggressor. The relative growth rate of most plants is rarely reduced by shading until the light intensity falls to 50–60% daylight (Blackman & Wilson, 1951*b*); this arises because the needs for the expansion of the leaf surface are met at a low level of total daily radiation (Milthorpe, 1959; Newton, 1958). Reduction in incident radiation, however, decreases the rate of growth of the root system and thereby accentuates the deficiency in nutrient supply. Light then becomes a second factor in competition, chiefly because it influences the rate of root extension through supply of assimilate to the root and this in turn affects the rate of nutrient uptake. Although available evidence is insufficient to determine the quantitative contribution of each factor to the growth of the suppressed species at all stages, it suggests that nutrient supply rather than light plays the major role. That is, greater growth of the less-successful competitor would be expected from an enhanced nutrient supply than from increased radiation incident on all its leaves. In the earlier stages, competition for nutrients appears to arise directly from the relative disposition of roots within the soil; subsequently light becomes more and more important through its influence on the supply of assimilate to the roots and hence the ability of the plant to absorb nutrients.

The influences of species on each other may also change because of differences in growth habits. In the last stages of the association between barley and persicaria there was a dramatic change in the relative growth of the two species. The cessation of root growth and mineral uptake and the senescence of leaves of barley after flowering improved the supply of both light and nutrients to persicaria. This species responded by producing branches from the upper axillary buds and was able to produce many ripe seeds by the time that the cereal was harvested. This response was greater the lower the plant density.

Although persicaria had little influence on the total dry weight of barley it decreased tiller production (cf. Part I, figs. 6, 7). Blackman & Templeman (1938) similarly found that reduction in yield of cereals by *Sinapis arvensis*, *Papaver rhoeas* and *Raphanus raphanistrum* was largely attributable to a decrease in the number of fertile shoots. The competition of barley with *Spergula arvensis* and *Matricaria inodora* (Mann & Barnes, 1945) and of wheat and oats with *Chondrilla juncea* (Myers & Lipsett, 1958) was also attributed to competition for nitrogen, the decreased tillering arising from a lower internal concentration of nitrogen. Although nitrogen has long been established as one of the main factors controlling branching (Gregory, 1937), light is also involved (Gregory & Veale, 1957), and some evidence from the present experiment suggests an effect of light. Although no analyses were made, the plants with reduced tillering had the appearance of 'high nitrogen' plants. Further, the subordinate plants for which the dry weights are given in Table 4 had the following tiller numbers: no competition 10·8, competition for light 6·5, competition for nutrients 4·8, competition for light and nutrients 6·2. Donald (1958) obtained very similar results for the tiller numbers of the aggressor species of two grasses, the numbers for treatments in the same order being 69, 60, 54 and 66. He also measured nitrogen uptake, and his data show that the relation between tiller number (at day 105) and nitrogen concentration (at day 74) was exactly the same in full light as where there was competition for light. The apparent influence of competition for light in Donald's and in our experiments therefore probably arose from differences in internal nitrogen concentration.

One general remark on interpretation of the interplay of environmental factors in competing systems may be added. In his experimental analysis Donald (1958) develops some concepts that appear only to lead to greater difficulty in understanding these very complex systems. He advances two axioms (p. 432): 'Firstly, there is not only a progressively decreasing share of the environment available to the suppressed species, but also a decreasing capacity by the suppressed species to make use of the environment available to it. Secondly, there is an interaction involved not only when there is competition for two factors, but also when there is competition for a single factor'. The first he illustrates by the situation where there is competition for nutrients only, there being direct effects due to the intrusion of the aggressor into the root environment of the suppressed species. The depletion of the nutrient supply to the latter leads to reduced leaf growth and for this reason only it intercepts less light. Therefore, he says, *its capacity to exploit the light*

environment is reduced. This is simply another way of stating that growth is reduced by shortage of nitrogen. There is no response on the part of the plant which reflects this postulated 'reduced capacity to exploit an abundant supply of one or more *other* factors'—the concept is purely abstract. The second axiom, which is a corollary of the first, is also unrealistic. As the reduced nutrient supply to the suppressed species is the cause of its reduced capacity to exploit the light available, it is difficult to understand what is meant by the postulated interaction—or indeed if such really exists.

Interactions between various environmental factors arise with the changing degree of association between the roots, stems and leaves of the component plants. Competition for one factor (such as nitrogen) leads to a shortage of that factor to the suppressed species. The consequent reduction in growth of the shoot causes it to be shaded more rapidly by the aggressor. That is, a decrease in the level of one factor in the immediate environment of the less successful competitor may result in the more rapid reduction of the level of the other environmental factors available to it. This in turn can lead directly to a further reduction in growth. The most valuable approach therefore would appear to involve a detailed *exploration* of the light and nutrient environments of the component species—that is, extending the studies of Black (1958) and Davidson & Donald (1958) relating to light to include the nutrient supply to each component species—and *explanation* of the influences of these environments in terms of the response of the main plant tissues (such as branching, rates of leaf production and expansion and rates of root growth).

I am particularly grateful to Prof. F. L. Milthorpe for discussions and suggestions during this study and in the preparation of this paper, to Mr L. Heathcote and Mr R. Dover for technical assistance and to Miss E. Lord for much help with the statistical analyses.

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OBSERVATIONS ON THE EFFECTS OF THE PARASITIC NEMATODES *DITYLENCHUS MYCELIOPHAGUS*, *APHELENCHOIDES COMPOSTICOLA* AND *PARAPHELENCHUS MYCELIOPHTHORUS* ON THE GROWTH AND CROPPING OF MUSHROOMS

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(With 22 Text-figures)

The parasitic nematodes *Ditylenchus myceliophagus*, *Aphelenchoides composticola* and *Paraphelenchus myceliophthorus* severely damage mushroom mycelium and, in great numbers, reduce or even prevent cropping. The relationships between fluctuation of nematode population, state of the mycelium, duration of cropping and the yield of mushrooms are illustrated in a series of figures.

Cairns & Thomas (1950), Thomas & Mitchell (1951), Cairns (1952*a*), Moreton (1953), Wood (1955) and Blake & Conroy (1959) described, in general terms, the effects of nematodes on the production of mushrooms (*Agaricus hortensis* Cooke). Newstead (1906) first reported the destruction of mushroom crops by *Ditylenchus* sp.; Lambert, Steiner & Drechsler (1949) observed the feeding of *Ditylenchus* sp. on mushroom hyphae, and Bovien (1951) identified the species as close to, and perhaps identical with, *Ditylenchus destructor* Thorne. Seinhorst & Bels (1951) showed that nematodes from mushroom compost behaved similarly to *D. destructor* from potato in attacking both potato tubers and mushroom mycelium on agar plates. Unable to find morphological differences, they identified the nematodes as *D. destructor*.

Cairns (1952*a*) stated that species of *Ditylenchus*, *Aphelenchus* and *Aphelenchoides* fed on, injured and eventually destroyed mushroom hyphae. Cairns also (1952*b*), working on the species of *Ditylenchus* attacking mushroom mycelium in U.S.A., showed that the fourth-stage larvae are capable of anabiosis. He discussed the relationships of this eelworm and its control by heat in laboratory experiments (1954*a*) and showed, in laboratory cultures, the varying effects of temperature on population increase and on pathogenicity to mushroom mycelium (1954*b*). Cairns (1955) later showed that aseptic specimens damaged and ultimately destroyed mushroom mycelium. He regarded (*in litt.*) this species of *Ditylenchus* in the U.S.A. as new. Goodey (1958*b*) described it as *D. myceliophagus*, after it had been partly differentiated by Paesler (1957) from *D. destructor* without being named.

Moreton, John & Goodey (1956) reported briefly on the destruction of mushroom

mycelium by *Aphelenchoides* sp. and on the rate of population increase under experimental conditions, and Franklin (1957) described this species as *Aphelenchoides composticola*.

Another nematode found destroying the mycelium in compost was *Paraphelenchus myceliophthorus* (Goodey, 1958*a*, 1959). *Aphelenchus avenae* has also been found occasionally in mushroom compost; on agar plates it attacks the mycelium vigorously.

Mushroom compost usually contains, associated with the above spear-bearing nematodes parasitic on the mycelium, various others usually regarded as saprobes. Brief mention is made of these, as they occurred in the experiments.

EXPERIMENTS

Experiments were made to find how the parasitic nematodes *Ditylenchus myceliophagus* J. B. Goodey, 1958, *Aphelenchoides composticola* Franklin, 1957, and *Paraphelenchus myceliophthorus* J. B. Goodey, 1958, affect mushroom mycelium and the production of sporophores. The changes in population of the parasitic and associated saprobic nematodes were also estimated.

The experiments started in 1952 when it was considered that *Ditylenchus myceliophagus* was a biologic 'race' of *D. destructor*, and it was hoped that they might help to separate the mushroom 'race' from the potato 'race' by a better understanding of their behaviour. The part of the work dealing with the relation of the potato 'race' to mushroom mycelium has already been published (Goodey, 1956).

METHODS AND MATERIALS

The preliminary experiments were made in a cellar but plaster moulds (*Scopulariopsius fimicola* and *Papulospora byssina*), vert de gris (*Myceliophthora*) and bubble disease (*Mycogone*) became rife and necessitated the making of a chamber that could be properly cleaned between experiments. This was approximately 5 ft. long by $2\frac{1}{2}$ ft. high by $2\frac{1}{2}$ ft. wide and held six wooden trays. The inner surfaces of the chamber were painted with 'Dentolite', a fungistatic and bacteriostatic emulsion paint. Between each experiment the trays were removed and they, and all the inner surfaces of the box, were washed with weak formaldehyde solution. With these precautions no fungi were seen growing on the inner surfaces of the box and occasional spots of plaster mould did not carry over from one experiment to another. The temperature of the box was maintained thermostatically and the atmosphere kept moist by water placed in a tray over the heaters near the base of the chamber.

Each wooden tray held fifteen, 5 in., plastic, plant pots, which were steam-sterilized before use and between each experiment. 300 g. of peak-heated compost, free from nematodes, were put into each pot and inoculated with either a small piece of moist, white, mushroom spawn or a few pieces of grain spawn. The incubation box was held at 70° F. for about 10 days to ensure that the mycelium ran

throughout the compost. Each pot was then cased with 200 g. of soil to give a layer about 1 in. thick. This casing soil consisted of:

Sterilized loam	2 buckets
Peat	1 bucket
Sieved, old, boiler ashes	1 bucket
Ground limestone	1, 6-in. potful

After casing, the temperature of the chamber was dropped to 65° F.

Inoculation with nematodes was done either when the spawn was introduced into the pots or when casing soil was added. *Ditylenchus myceliophagus*, *Aphelenchoides composticola*, *Paraphelenchus myceliophthorus* and *Panagrolaimus* sp. were extracted from infested compost, *Ditylenchus dipsaci* from 'tulip-rooted' oats and *D. destructor* from diseased potato tubers on a Seinhorst Mistifier (Goodey, 1957) and the requisite number inoculated on to the compost in a little water. Pots of compost were randomized before nematode inoculation and were kept to constant

TABLE 1. *Detailed summary of data relating to the setting up and progress of Exps.* 1–8 *in which the effects of* Ditylenchus myceliophagus, D. destructor, D. dipsaci, Aphelenchoides composticola *and* Panagrolaimus *sp. on the growth and cropping of mushrooms were tested.*

Exp. no.	Nematode species used for inoculum	Numbers of nematodes inoculated per 100 g. pot contents	Replication	Days from starting experiments: Nematodes added	Pots cased	Cropping started	Results given in[1]
1	*D. myceliophagus*, *D. destructor*	0, 130, 650, 3250, 16250	4	20	20	41	Fig. 1
2	*D. myceliophagus*, *D. dipsaci*	0, 26, 130, 650, 3250	5	27	27	50	Figs. 2, 3
3	*D. destructor*	0, 83, 833, 8333, 83333	10	11	11	26	Fig. 4
4	*D. myceliophagus*	0, 70, 700, 7000	20	11	11	28	Figs. 5, 11–13
5 *a*	*D. myceliophagus*	3, 30	5	11	11	18	Figs. 6, 14–17
b	*D. myceliophagus*	3, 30	15	11			
	Control	0	10	—	—	—	—
6	*A. composticola*	0, 1, 3*, 30	10	1	12	27	Figs. 7, 18–21
	Panagrolaimus sp.	0, 3*					
7	*A. composticola*	0	8	1	14	29	Figs. 8, 22
		†0·2	10				
		2	60				
8	*Panagrolaimus* sp., *A. composticola*, *D. myceliophagus*	2	10	1	13	28	Fig. 9

* Hand-picked specimens. † = 1 gravid ♀ per pot.

weight by watering every other day before casing and twice weekly afterwards. Once cropping began, allowance was made for the weight of mushrooms removed.

Weekly observations were made on the state of the mycelium in soil and compost, and the numbers of nematodes in each estimated. The duration of growth and the number and weight of mushrooms produced were also recorded (each sporophore was weighed with the stipe cut off at the annulus). The maximum capacity of the chamber was ninety pots, which limited the number of examinations possible. The first were usually made after casing and examination continued for 11 or

12 weeks; this period was always longer than that during which cropping continued in the control pots. On each occasion a set of pots was taken, the casing soil removed, examined, mixed and weighed. The compost was similarly treated. From each lot, a sample of 100 g. had its nematode burden extracted for 48 hr. on a Seinhorst mistifier, which was known to give a 21% extraction of nematodes from compost and 59% from soil after 48 hr. After extraction the nematodes were counted in paired, aliquot samples with, where possible, at least 100 specimens in each, in Fenwick's modification of McMaster counting slides (Goodey, 1957). Counts were adjusted to allow for the extraction efficiency of the apparatus. The saprobic nematodes present were estimated separately. Where possible, data on the cropping and average yield of mushrooms per pot were analysed statistically, but often this could not be done because pots were examined before cropping had finished; the results from such tests are based on pots in which cropping was complete.

Table 1 gives the detailed plan and progress of eight experiments over a period of $3\frac{1}{2}$ years.

RESULTS

A series of graphs and histograms (Figs. 1–22) summarize the results. The following are brief comments on each experiment.

Experiment 1. Although diseases, already referred to, prematurely killed many mushrooms in this experiment done in a cellar, there were enough results to indicate that, judged by duration of cropping, *D. myceliophagus* considerably damaged the mycelium whereas *D. destructor* did little damage.

Experiment 2. *D. myceliophagus* significantly shortened the duration of cropping. Rates of nematode inoculation and average yields of mushroom per pot also showed some significant differences. *D. dipsaci* had no significant effect.

Experiment 3. At the highest inoculation rate *D. destructor* slightly but insignificantly depressed the duration of cropping and the average yield of mushrooms per pot. Little, if any, damage was caused to the mycelium, and none of the female nematodes recovered was gravid, suggesting that reproduction had not taken place.

Experiment 4. *D. myceliophagus* affected yield and duration of cropping significantly.

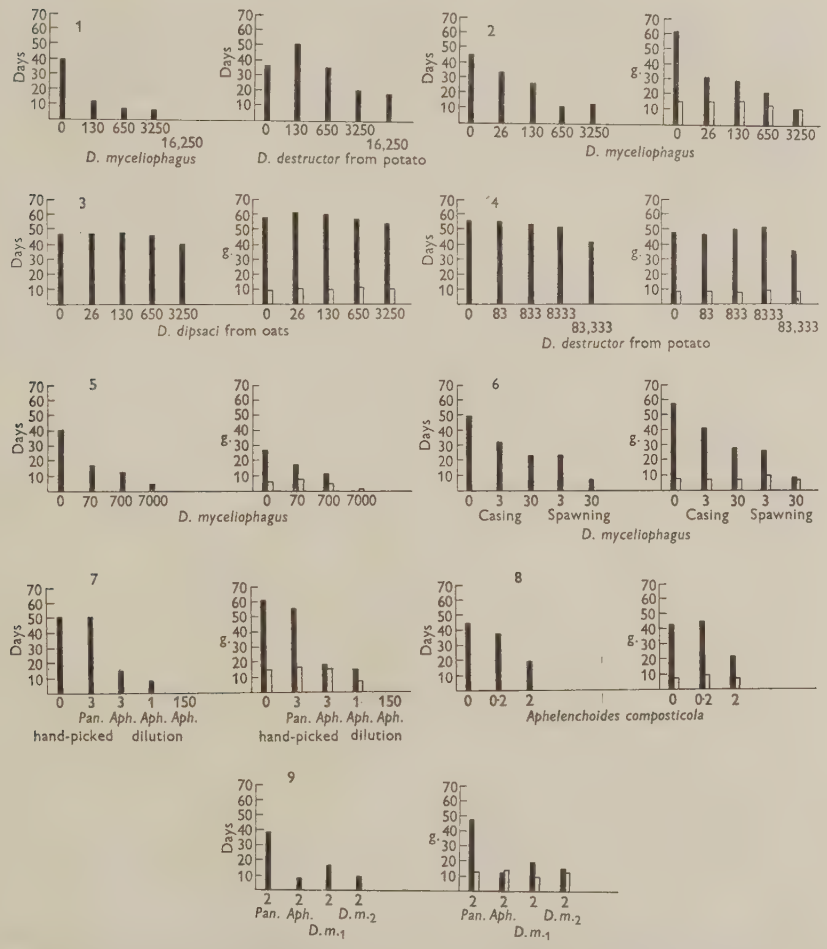
Experiment 5. Inoculation at spawning was compared with inoculation at casing for *D. myceliophagus*. The former shortened duration of cropping and lowered the mean yield of mushrooms per pot but the average weight of individual mushrooms was little affected.

Experiment 6. *Aphelenchoides composticola* inoculated at spawning shortened cropping to fewer than 6 days; indeed, cropping stopped before the weekly examination of pot contents began. Pots inoculated with *Panagrolaimus* cropped as long as the controls.

Experiment 7. The progeny from ten *Aphelenchoides composticola* considerably shortened duration of cropping and even the progeny from one appears to have had some effect. After 15 weeks, the individual specimens of *A. composticola* usually produced populations of just over 500,000 eelworms per pot.

Experiment 8. This small-scale experiment compared the effects of *Panagrolaimus* with *Aphelenchoides composticola* and with *Ditylenchus myceliophagus* from two different sources. It confirmed that *Aphelenchoides composticola* is more destructive than *Ditylenchus mycelophagus*.

Experiment 9. The effects of *Paraphelenchus myceliophagus* were tested in 1 lb.



Figs. 1–9. The effect of *Ditylenchus myceliophagus*, *D. destructor*, *D. dipsaci*, *Aphelenchoides composticola* and *Panagrolaimus* sp. on the duration of mushroom cropping, mean yield and average weight/pot. Abscissae: inoculation rate, nematodes/100 g. Left half of each histogram: duration of cropping. Right half of each histogram: ■, mean yield of mushrooms/pot; □, average individual weight of mushrooms/pot. *N.B.* In Fig. 1 the right half refers to duration of cropping.

jam jars filled with 130 g. of compost. After 8 weeks the mycelium in the inoculated jars had almost disappeared but there was still a dense growth in the controls. The population of *P. myceliophthorus* had then risen from eight per 100 g. of compost to 443,700, and after a further 5 weeks stood at 71,800.

Discussion

These results provide a detailed picture showing the response of the mushroom mycelium to attack by the various parasitic and saprobic nematodes. In the controls the mycelium ramified throughout the compost and casing soil and remained in

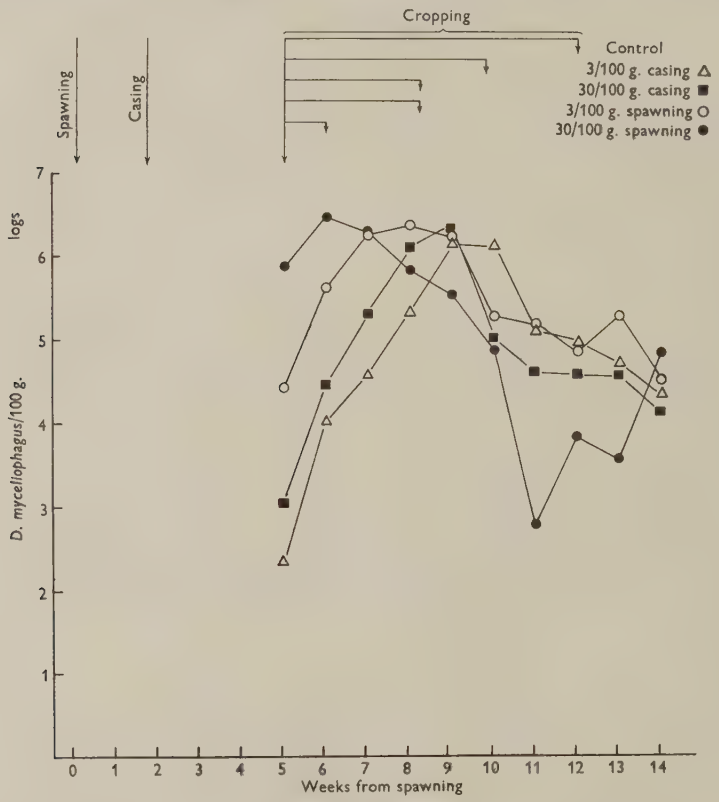
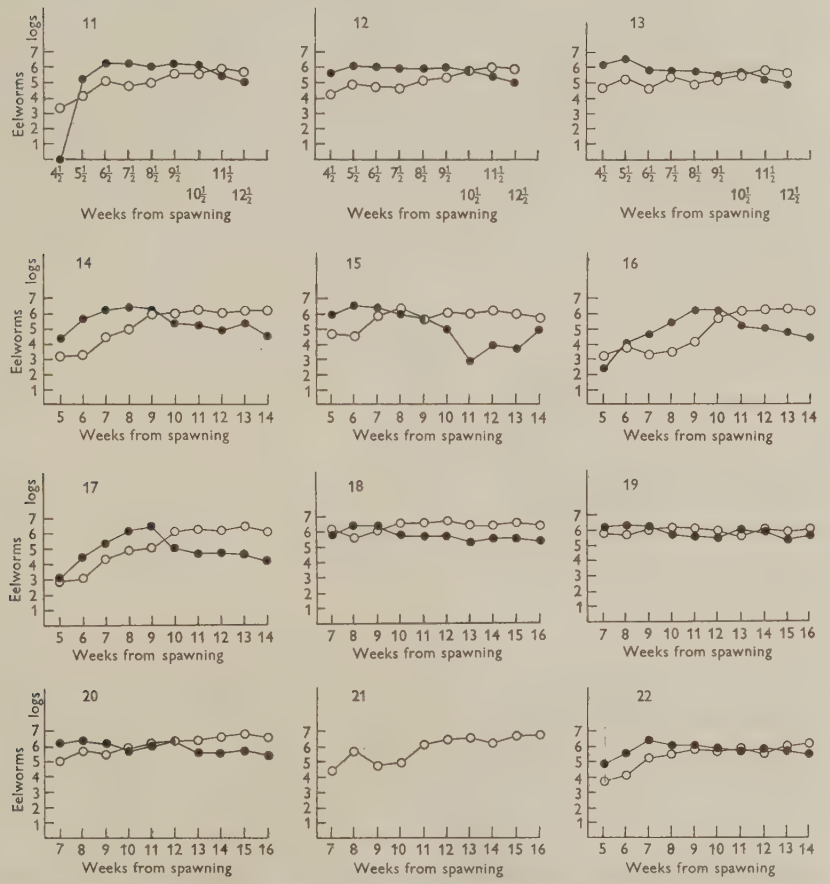


Fig. 10. The relationship between crop duration of mushrooms and changes in populations of *D. myceliophagus* following inoculation of compost at spawning or casing.

good condition for 15 weeks or so. Mycelium developed similarly in pots infested with pathogenic eelworms but, whether inoculation with eelworms was at spawning or casing, it was gradually destroyed. The first sign of attack was a deterioration of the mycelium at the interface of compost and casing soil where eelworms were

introduced. In both compost and soil, the mycelium first became sparse and stringy and then disappeared. The mycelial destruction was reflected in the death of the first young button mushrooms and the failure to produce more.



Figs. 11–22. Population variation of *Ditylenchus myceliophagus*, *Aphelenchoides composticola* (parasites) and *Panagrolaimus* sp. (saprobe) in mushroom compost in relation to time and to different inoculation rates of nematodes at spawning or casing. Figs. 11–17, *D. myceliophagus*; Figs. 18–20, 22, *A. composticola*; Fig. 21, *Panagrolaimus* sp. ●, Parasitic spp.; ○, saprobes. Figs. 11–13. 70, 700, 7000 *D. myceliophagus*/100 g. at casing respectively. Figs. 14–16. 3, 30, 3 *D. myceliophagus*/100 g. at casing respectively. Fig. 17. 30 *D. myceliophagus*/100 g. at casing. Fig. 18. 1 *A. composticola* (by dilution)/100 g. at spawning. Fig. 19. 30 *A. composticola* (by dilution)/100 g. at spawning. Fig. 20. 3 *A. composticola* (hand-picked)/100 g. at spawning. Fig. 21. 3 *Panagrolaimus* (hand-picked)/100 g. at spawning. Fig. 22. 2 *A. composticola* (hand-picked)/100 g. at spawning.

The rate at which the mycelium was destroyed in the infested pots varied with the number of nematodes added and the time at which they were added. Table 2 shows results from some of the experiments. These rates of destruction cannot be closely compared with those reported by Cairns (1954*b*), also caused by *Ditylenchus myceliophagus*, because his data are in a different form and there is no indication of the numbers of nematodes he added, but, at 65° F. (the temperature in my experiments), Cairns reported total destruction of mycelium in about 7 weeks.

A more general picture of the course of nematode attack on mushroom mycelium in compost, which correlates duration of cropping with the changes in population density of parasitic and saprobic nematodes, and the appearance of the mycelium in the compost, can be given.

TABLE 2. *Rate of destruction of mushroom mycelium by* Ditylenchus myceliophagus *and* Aphelenchoides composticola

Exp. no.	Nematode species	Inoculation rate nematodes/100 g.	Inoculation time*	Days from spawning for total destruction of mycelium
4	*D. myceliophagus*	7000	C	46
4	*D. myceliophagus*	700	C	60
4	*D. myceliophagus*	70	C	67
5	*D. myceliophagus*	30	C	56
5	*D. myceliophagus*	3	C	70
6	*A. composticola*	30	S	53
6	*A. composticola*	3	S	74
6	*A. composticola*	1	S	81
6	*Panagrolaimus* sp.	3	S	Still present at 102
7	*A. composticola*	2	S	84

*C = Casing; S = spawning.

Mycelium spreads, from the original inoculum, throughout the compost in about 10 days. Casing soil is added at this time and the mycelium grows through it, finally producing sporophores at about 4 weeks from starting. Cropping may go on for 7 to 8 weeks on the control pots but it is shortened in pots inoculated with parasitic nematodes, the period depending on the size of the initial inoculum. When this is great, particularly when inoculation is at spawning, the mycelium may be destroyed so rapidly that no sporophores are produced. The number of parasitic nematodes in the compost increases during the early weeks and reaches a peak just about the time that cropping ceases: high initial numbers reach an early peak and low a later one. After reaching this peak, usually in the lower millions per 100 g. of compost, the numbers decrease. The mycelium in the compost begins to disappear at about the time the peak numbers of parasitic eelworms appear, and the mycelium is usually completely destroyed 10 days to a fortnight later. A few weeks after the peak of parasitic nematodes, a second peak of saprobic nematodes is reached. These have built up more gradually; their peak numbers vary but may be as high as those of the parasites. The population pattern shown by the saprobes may include several peaks and troughs during the weeks after the first peak, but eventually the numbers

also diminish. Presumably as the mycelium is destroyed by the parasites the food supply for bacteria is greatly increased. The bacteria present in the compost therefore multiply greatly and in their wake the population of saprobes increases. It seems likely from the work of Hirschmann (1952) that many saprobic nematodes require the protein of living bacteria for breeding.

As an example of this general picture of the sequence of events, data from Exp. 6 are presented in Fig. 10, which shows the relationships of spawning, casing, cropping, and the fluctuation of nematode populations with time.

I thank Miss Mary Muir and Mr David Hooper for their help in the conduct of the experiments. Several members of the National Agricultural Advisory Service and particularly Mr B. D. Moreton helped by supplying nematode-infested mushroom compost at various times. Mr Stewart Wood of Aylesbury was most generous in supplying peak-heated compost, and Messrs Darlington and Sons Ltd., Worthing, were similarly helpful in giving cultures of grain and moist white spawn. To Mr F. C. Wood, of the last named organization, I am deeply indebted for continual, friendly advice and comments on mushroom growing, without which this work would not have contained the particular interest it did.

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